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PROSTATE CANCER DIAGNOSTICS –

complications and ways to reduce unnecessary biopsies

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PROSTATE CANCER DIAGNOSTICS –

complications and ways to reduce unnecessary biopsies

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Till

Martina, Hedvig och Sigrid

”Knowledge without follow-through is worse than no knowledge at all. because if you're guessing and it doesn't work out you can just say, shit, the gods are against me. but if you know and don't do, you've got attics and dark halls in your mind to walk up and down in and wonder about. this ain't healthy, leads to unpleasant evenings, too much to drink and the shredding machine.”

Charles Bukowski

ABSTRACT

Introduction: Prostate cancer is the most common form of cancer among men in Sweden. There has been a rapid increase in the incidence rate of prostate cancer following the introduction of PSA testing and, today, more than 1800 men are diagnosed with the disease annually in Stockholm, Sweden. This increased testing has, however, not led to any significant reduction in the mortality of prostate cancer. There is no official screening programme for prostate cancer in Sweden, however, more than 60% of men above the age of 60 have undergone a PSA test in the last 5 years. What is less known is what proportion of men undergo a prostate biopsy after a PSA test and within what time frame. The majority of men undergoing a prostate biopsy are not diagnosed with a prostate cancer. In a setting where the PSA test had a better specificity these men would not have to undergo a prostate biopsy. To perform a prostate biopsy is not without risks. Serious infectious complications following prostate biopsies have been reported to be increasing in other parts of the world. The serious infectious complication rate in Stockholm, following a prostate biopsy, is not known.

Aims: To investigate if genetic markers, SNPs, can be used as a complement to PSA to predict which men with a PSA <10 ng/mL need to undergo prostate biopsies. To explore the prostate biopsy rates and results in Stockholm and to investigate when PSA testing leads to prostate biopsies and to what extent these prostate biopsies cause side effects in terms of severe infections.

Material and Methods: In *Study I*, 8088 men were identified who underwent at least one prostate biopsy in Stockholm between 2005 and 2007. Those alive and younger than 80 years of age, were invited to donate blood and fill out a questionnaire. 2542 men were included in the analysis when restricted to age less than 80, alive at time of invitation, valid PIN, and a PSA <10 ng/mL. In *Study II*, 860 men aged 50 to 69 years with a PSA of 1-3 ng/mL without a history of prostate cancer or previous prostate biopsies were invited to undergo a prostate biopsy. 172 men were stratified into low-, intermediate- and high-risk groups according to their genetic score and then underwent a prostate biopsy. In *Studies I and II*, a genetic score, based on the known SNPs associated with a risk of prostate cancer at the time of the study in combination with PSA and other predictive factors, was created and used in a prediction model to enhance specificity in men with a PSA<10 ng/mL and sensitivity in men with a PSA of 1-3 ng/mL. In *Study III,* men who had undergone at least one prostate biopsy in Stockholm from 2003 to 2012 were included. Biopsies done in 2003 were acknowledged but not included in the analysis. Migration data was used for population analysis. Data from 38 800 biopsies was analysed. Main outcome in the study was time from PSA test to prostate

biopsy. In *Study IV*, prostate biopsies (n=44 047) done from 2003 to 2012 were included and linked by the use of PIN to microbiological data resources to identify blood cultures taken and available biograms. The main variable studied for outcome was year of biopsy. Logistic regression and time to event were used to address associations. The net reclassification index was used to evaluate the predictive performance of the genetic risk score. In all the studies men were linked to several health registers, such as the Swedish Cancer Register, the National Prostate Cancer Register, the Swedish Cause of Death Register, the National Patient Register, and the Total Population Register.

Results: In *Study I*, up to 23% of the prostate biopsies could have been avoided by using a genetic risk score in combination with age, family history, PSA and f/t PSA. The proportion of missed cancers would be between 5.8 and 12% depending on the risk cut-off used. The proportion of aggressive cancers missed would be between 3.3 and 8.3%. In *Study II*, the proportion of cancers diagnosed in the low-, intermediate- and high-risk groups was 18, 28 and 37 %, respectively ($p<0.05$). A borderline significant trend was seen between a higher genetic risk score and the risk of an aggressive prostate cancer. In *Study III,* 58 and 45% of men in aged 50-59 and 60-69 years of age, respectively, with a PSA between 4 and 10 ng/mL underwent a prostate biopsy within one year of the PSA test. For men with a PSA >10 ng/mL the proportion was 67 and 58% respectively. One out of eight men with an advanced prostate cancer had a first known PSA of >4 ng/mL more than 6 months prior to their diagnosis. In *Study IV*, the proportion of men with a positive blood culture within 30 days of the prostate biopsy in 2003 was 0.38 and 1.14% in 2012. Year of biopsy was highly significant as a risk factor for undergoing a blood culture and was robust both in the simple - and the adjusted analysis. Young age and low PSA values were associated with a risk of undergoing a blood culture. Men with a high Charlson Comorbidity Index had an increased risk of undergoing a blood culture. Bacteria resistant to common prophylactic antibiotics were more frequently found in blood cultures in the later years of the study than in the early years.

Conclusion: A genetic risk score can be used to enhance the sensitivity and specificity of PSA in men undergoing an investigation for prostate cancer. By reducing the number of unnecessary biopsies the number of men suffering from severe infectious complications will be reduced as well as the number diagnosed with a low-risk prostate cancer. The proportion of relatively young men not undergoing a prostate biopsy within one year of the PSA test, although their result was pathological, was surprisingly high. One way to solve this problem would be to introduce a structured follow-up after PSA testing.

LIST OF SCIENTIFIC PAPERS

I. **Polygenic risk score improves prostate cancer risk prediction: results from the Stockholm-1 cohort study**
M. Aly, F. Wiklund, J. Xu, W. B. Isaacs, M. Eklund, M. D'Amato, J. Adolfsson, H. Grönberg
European Urology 60(2011) 21-28

II. **A genetic risk score can identify men at high risk for prostate cancer among men with prostate-specific antigen of 1-3 ng/mL**
T. Nordström, M. Aly, M. Eklund, L. Egevad, H. Grönberg
European Urology 65(2014) 1184-1190

III. **Delayed diagnosis of prostate cancer – result of unstructured prostate cancer testing?**
M. Aly, C. E. Weibull, M. Clements, P. Dickman, J. Adolfsson, E. Näslund, H. Grönberg
(submitted)

IV. **Rapid increase in multidrug-resistant enteric bacilli blood stream infection after prostate biopsy – a 10-year population cohort study.**
M. Aly, R. Dyrdak, T. Nordström, C. E. Weibull, S. Jalal, C. G. Giske, H. Grönberg
(submitted)

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LIST OF ABBREVIATIONS

AUC	Area Under the Curve
CCI	Charlson Comorbidity Index
CR	The Swedish Cancer Register
DNA	Deoxyribonucleic Acid
DRE	Digital Rectal Examination
ERSPC	European Randomized Screening trial for Prostate Cancer
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
MRI	Magnetic Resonance Imaging
NPCR	National Prostate Cancer Register
NPR	National Patient Register
OR	Odds Ratio
PIVOT	Prostate Cancer Intervention versus Observation Trial
PLCO	Prostate, Lung, Colon and Ovarian Cancer Screening trial
PSA	Prostate Specific Antigen
RALP	Robot-Assisted Laparoscopic Prostatectomy
RNA	Ribonucleic Acid
RRP	Retropubic Radical Prostatectomy
SCOD	Swedish Cause of Death Register
SPCG	Scandinavian Prostate Cancer Group
PIN	Personal Identification Number
TMA	Tissue Micro Array
TPR	Total Population Register
TUR-B	Transurethral Resection of the Bladder
TUR-P	Transurethral Resection of the Prostate

1 OVERVIEW OF THESIS

	Aim	Subjects and Methods	Results and Conclusion
I	To investigate if a genetic risk score based on SNPs can be used in a prediction model to avoid unnecessary prostate biopsies.	Men in Stockholm who had undergone a first known prostate biopsy from 2005 to 2007 with a PSA <10 ng/mL. Logistic regression and prediction. NRI.	Using a genetic model, 12 to 23% of the prostate biopsies would be avoided, 6-12% of cancers would be missed, and 3.3-8.3% of the aggressive cancers would be missed. A genetic risk score can be used to avoid unnecessary prostate biopsies in men with moderately elevated PSA.
II	To investigate if a genetic risk score based on SNPs can be used to identify men with a high risk of prostate cancer although their PSA is low.	Men, aged 50-69 years, in Stockholm with no prior history of prostate biopsies and prostate cancer with a PSA of 1-3 ng/mL. 10-12 core biopsies were done using ultrasound-guided technique. Logistic regression analysis, NRI.	Prostate cancer was detected in 27% of the 172 men undergoing a prostate biopsy. Stratified by their genetic risk, prostate cancer was found in 18, 28 and 37% of men with a low, intermediate and high genetic risk score, respectively. A genetic risk score can be used to identify men with a high risk of prostate cancer although their PSA is low.
III	To investigate the follow-up of pathological PSA values in men living in Stockholm between 2004 and 2012.	Men who were living in Stockholm between 2004 and 2012 and underwent a core biopsy of the prostate. Background population in Stockholm Survival analysis. Population-based.	67 and 58% of men aged 50-59 and 60-69 years, respectively, with a PSA >10 ng/mL undergo a prostate biopsy within one year of the PSA test. One out six men diagnosed with an advanced prostate cancer had a pathological PSA more than 6 months prior to their diagnosis The situation in Stockholm, with an unstructured follow-up of pathological PSA testing, is suboptimal.
IV	To investigate if the rate of severe infectious complications has increased during the last 10 years in Stockholm. To investigate if men undergoing a prostate biopsy have a higher mortality rate.	Prostate biopsies performed in Stockholm from 2003 to 2012. The men were linked to microbiological laboratories. Logistic regression analysis. Standard mortality rate. Population-based.	The proportion of men with symptoms suggestive of blood stream infection rose from 1.14% in 2003 to 2.31% in 2012. Mortality rates were not higher in men undergoing a prostate biopsy compared with Swedish men in general. The infectious complication rate has more than doubled in 10 years, which is likely attributed to an increase in multidrug-resistant bacteria. Patients and physicians have to be aware of this increase when deciding to perform a prostate biopsy.

2 BACKGROUND

2.1 EPIDEMIOLOGY

2.1.1.1 Incidence of prostate cancer in Sweden

During the last two decades the incidence rate of prostate cancer has increased substantially in Sweden. The incidence rate in 1970 was 71 new cases per 100 000 men and rose steadily to the mid-1990s when it reached 131. Thereafter, the rate increased faster and reached its maximum in 2009 when 229 new cases per 100 000 men were detected (Figure 1). The main reason for the incline in incidence is most certainly due to the increased testing by PSA, which was introduced in the 1990s. Prostate cancers that have been diagnosed in more recent years show a lower stage at detection compared with earlier diagnosed prostate cancers. The median age at diagnosis has decreased from 74 years in 1996 to 70 years in 2005 [1].

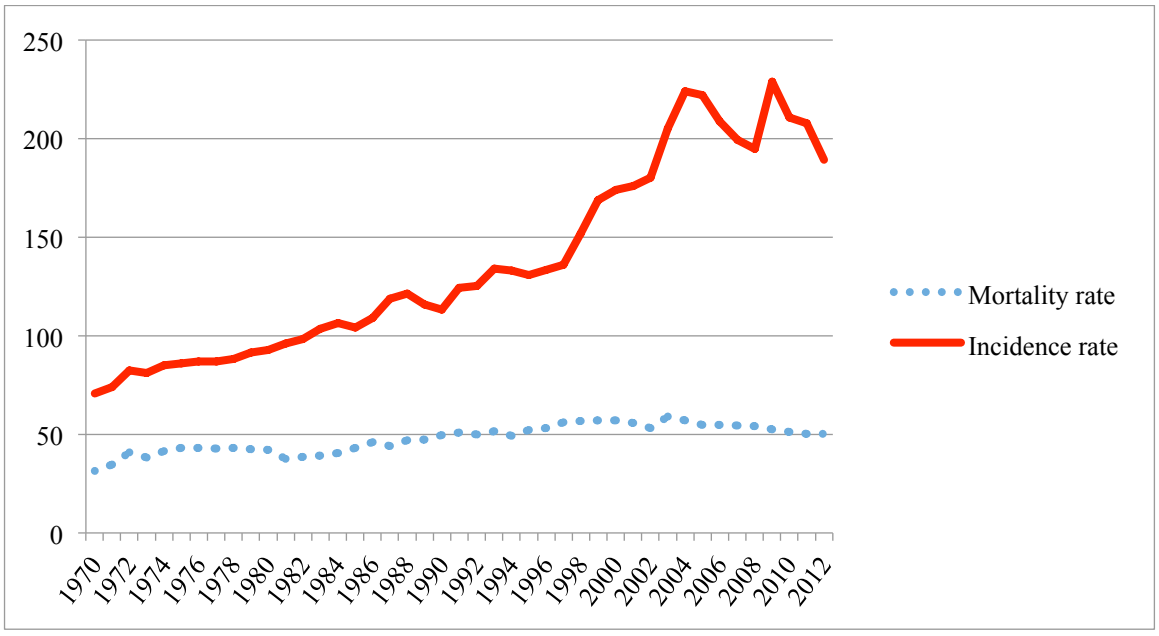

Figure 1.
Incidence and mortality rate for prostate cancer per 100 000 men in Sweden, crude rate. Incidence rate from Socialstyrelsen, statistikdatabasen, mortality rate from NORDCAN, (Accessed 20140921) [2,3].

2.1.1.2 Mortality

Scandinavian countries, together with North America, have among the highest mortality rates worldwide. Although the incidence of prostate cancer has increased fourfold in the last two decades in Sweden, the mortality rate has been relatively stable throughout the last four

decades. Although the disease today is detected earlier and at a lower stage it has not lead to a decrease in mortality, until recently. During the last five years the mortality rate has decreased slightly (Figure 1). The reduction in mortality witnessed over the last few years is probably an effect of the widespread PSA testing that began in the late 1990s [4].

Mortality in prostate cancer is very dependent on the grade of the disease. Men diagnosed with an aggressive disease will have an affected course of life whereas men with a non-aggressive disease will most likely succumb to other diseases, even without treatment [5-8]. Screening studies done to measure the effects of screening on prostate cancer mortality have been performed. Two large randomised studies were presented in 2009: the European study, ERSPC and an American trial called PLCO. The ERSPC trial showed a 20% relative risk reduction in mortality at nine years of follow-up whereas the American trial could not demonstrate any benefits of PSA screening [9,10]. These studies will be more extensively discussed in a later chapter.

2.2 RISK FACTORS

Prostate cancer is a multifactorial disease with no clear aetiology. No specific event is known to trigger the disease and no specific causes are known that influence its progression. Evidence exists that both genetics and the environment play a role in disease initiation and development. There are three established risk factors: age, family history and ethnic origin.

2.2.1.1 Age

Prostate cancer is relatively uncommon before the age of 50. The median age of diagnosis is 68 years with 63% being diagnosed after the age of 65 in the US. The median age for diagnosis in Sweden is 70 years of age [1,11].

Autopsy studies performed on men dying from other causes than prostate cancer have indicated that latent prostate cancer is common, and the prevalence increases with age. In men in their 4th decade of life, 8.8-27% harboured small foci of prostate cancer, for men in their 5th decade of life the proportion was 14.8-34%. For Asian men, these proportions are lower in younger ages but reach the same levels later in life. This is interesting since the mortality rates for prostate cancer are much higher for Caucasian than for Asian men [12-14].

2.2.1.2 Family history

For investigative purposes, prostate cancer can be divided into three groups: sporadic, familial and hereditary. Familial prostate cancer is defined as cancer in a man with more than one affected relative. Hereditary prostate cancer occurs in men with more than three affected relatives, i.e. men with three prior generations where prostate cancer has been diagnosed or in men with two or more close relatives diagnosed with the disease before the age of 55. Sporadic cancers occur in men with a negative family history [15].

The relative risk for men with an affected father is 2.17 times higher than for a man with an unaffected one, while the risk for a man with a brother diagnosed with the disease is 3.37. The relative risk for a man with more than two affected first-degree relatives is 5.08 [16].

2.2.1.3 Ethnic origin

There are great differences in age-standardised incidence rates for prostate cancer in different continents and countries. Northern Europe, the United States, Australia and New Zealand for example have among the highest age-standardised rates, reaching more than 100 new cases per 100 000 men per year. Countries in Asia have significantly lower rates: the rate for Japan, for example, is approximately 30 new cases per 100 000 men per year (Figure 2) [17].

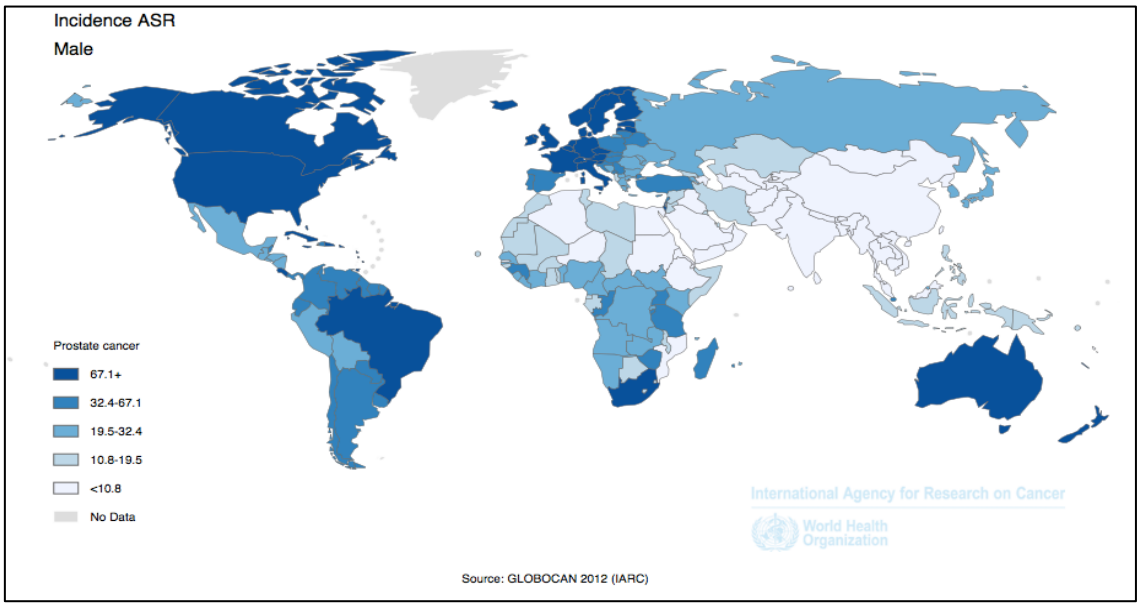

Figure 2.
Incidence of prostate cancer worldwide (Age-standardised rates) [17](copyright, webpage accessed 20140918).

Afro-American men in the United States have among the highest risk of all men, considerably higher than for Caucasian men.

An intriguing fact is that men who move from Japan to the United States increase their risk, and approach the risk of American men [18]. This suggests that not only genetic factors influence the risk of developing prostate cancer, but also external exposures such as environment and perhaps dietary factors contribute to the risk.

2.1 ANATOMY AND PHYSIOLOGY

The prostate is located in the male pelvis, circumflexing the urethra. It lies below the urinary bladder in front of the rectum. At the bottom of the pelvic floor lies the apex of the prostate, the urethra exits the prostate in the apical region and enters the penile structures. The vas deferens, which leads the sperms from the testicle to the lumen of the urethra, enters the prostate between the rectum and the prostate. The seminal vesicles are located at the base of the prostate, close to the bladder and in close abundance to the vas deferens.

The neurovascular bundle runs at the lateral surface of the prostate. The inferior vesicle artery, with its origin from the iliac internal artery, supplies the prostate with blood. The veins draining the prostate run in the same bundle and eventually enter the inferior vesicle vein which later drains into the inferior internal vein.

The nerves transmitting signals to accommodate erection also run in the neurovascular bundle; which is of clinical importance as these nerves are sensitive and might be traumatised during radical treatment of the prostate [19,20]. There is some evidence that nerves in this region also partially supply the urinary sphincter and, thus, play a role in urinary continence [21].

The prostate is a gland, and its growth is testosterone-dependent. Before puberty, the gland is the size of a cherry, but during puberty it grows to the size of a walnut weighing approximately 18 grams. For some men this growth continues throughout life, eventually causing a problem of micturition.

The main function of the prostate is to produce Prostate-Specific Antigen, which is an enzyme belonging to the serine protease group. Its main purpose is to liquefy the semen at the time of ejaculation making the sperms more motile – thereby increasing the chances of fertilising the egg.

The prostate is subdivided into different anatomical/histological zones with slightly different properties. For example, the inner middle region circumflexing the urethra, the transition zone, is predominantly responsible for an older man's difficulties to void as this part grows with age. The majority (85%) of the prostate cancers are found in the peripheral zone – where the dorsal parts are accessible per rectum for clinical staging (Figure 3) [22].

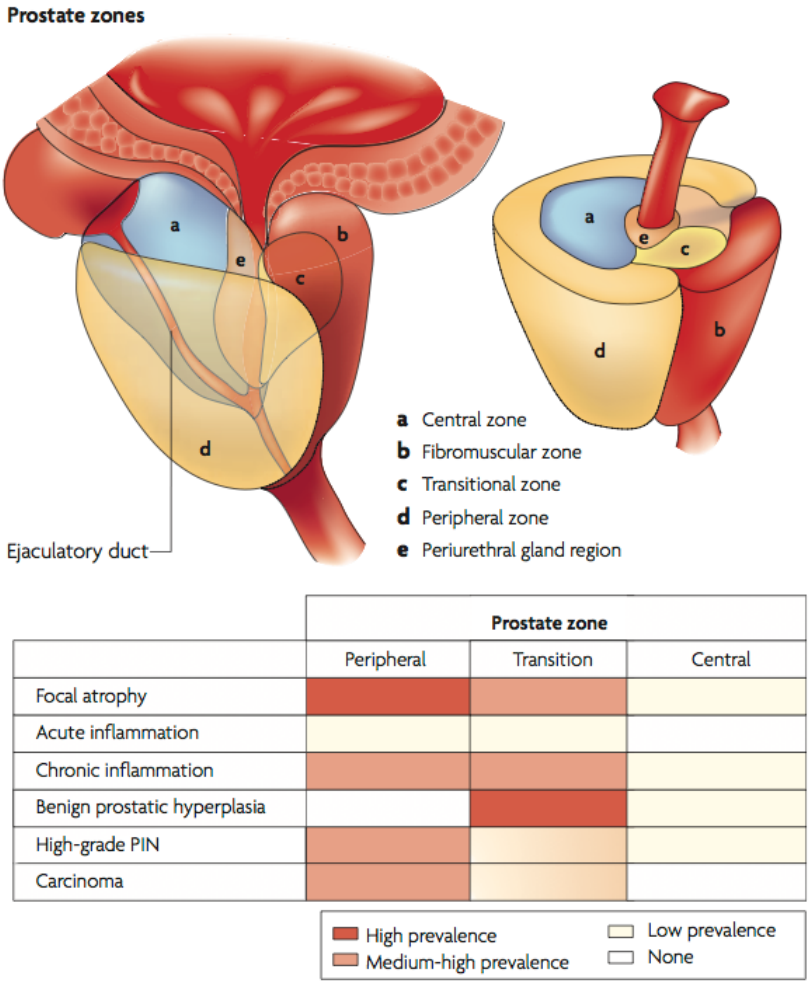

Figure 3.
The prostate gland and its zones and their predisposition to prostate diseases [23](reprinted with permission from Nature Publishing Group).

2.2 SYMPTOMS OF PROSTATE CANCER

In the very early stages of prostate cancer, the patient rarely complains of any symptoms. In the later stages some men may complain of problems emptying the bladder – but this is fairly uncommon. Most men with voiding problems do not have a clinically significant prostate cancer but a benign prostatic hyperplasia causing the symptoms.

In the advanced stage of prostate cancer men may complain of skeletal pain as a result of metastasises. Some may also present with hydronephrosis and renal damage as a consequence of the tumour pressing against the ureters hindering urine to pass normally from the kidney to the bladder. Pain in the lower abdomen is rarely related to prostate cancer.

2.2.1.1 Clinical findings

The first steps to take when meeting a patient with symptoms or anxiety about having a prostate cancer are to analyse the PSA and to palpate the prostate. If laboratory results or the DRE raise the suspicion of prostate cancer a histological or cytological evaluation is important. This information, together with DRE and PSA, can provide important prognostic information and also guide the physician whether or not to evaluate lymph node status by means of an MRI or bone metastasis by means of a bone scintigram.

2.3 DIAGNOSTIC MARKERS OF PROSTATE CANCER

An optimal diagnostic marker can differentiate men with a prostate cancer from those without the disease. A marker that at a certain threshold identifies all men with the disease is said to have a high sensitivity, whereas a marker with a high specificity correctly classifies those without the disease to a high degree. The optimal marker has both a high sensitivity and a high specificity. Unfortunately, no such marker has yet been found for prostate cancer. A number of markers have been described but only a few have reached everyday clinical practice.

2.3.1.1 Prostate-Specific Antigen

The discovery of PSA is the work of several researchers independent from each other. In 1960, Flocks identified species-specific prostate antigens. In 1964 Hara identified a prostate-specific antigen in the semen, which could be used in forensic medicine when investigating rapes. Ablin identified two antigens in the prostate, one was prostatic acid phosphatase and the other, which needed further investigation, was called prostate-specific antigen. In 1966 Hara published an article describing the γ-seminoprotein, which would later be proven to be PSA. In 1980, Papsidero succeeded in measuring PSA in serum. Building on the work of this early research Chu and colleagues were able to patent the discovery and identification of PSA in 1984. But it was not until 1987 when Stamey published an article in the New England

Journal of Medicine where he showed that the stage* of prostate cancer was related to the PSA level measured in serum. After this, it was concluded that PSA could be used as a tumour marker for prostate cancer (Figure 4). Stamey also showed that PSA was not measurable after a prostatectomy thereby making it useful as a tool to monitor disease progression [24,25].

Despite its name PSA is not prostate specific, other organs as well, such as normal prostate tissue and malignant breast tissue and adrenal and renal carcinomas may produce it, but concentrations in serum from these localities are not clinically relevant. PSA can be elevated not only due to prostate cancer but also due to other diseases such as benign prostatic hyperplasia or urinary tract infection [22].

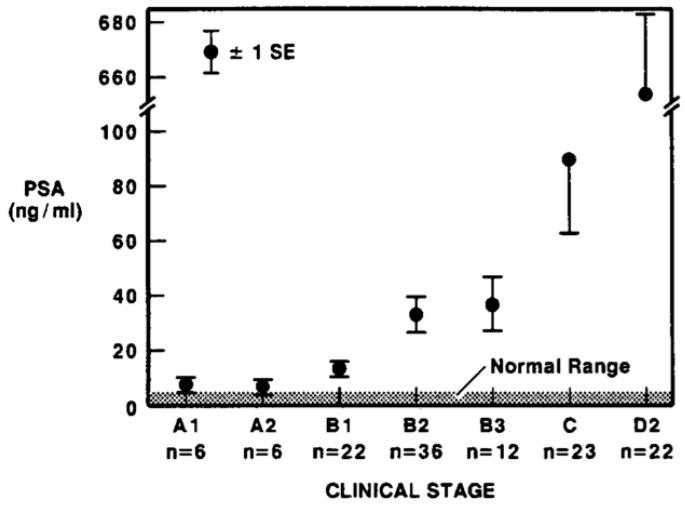

Figure 4.
Relation of the concentration of PSA to the clinical stage of prostate cancer in 127 patients (reproduced with permission from [25], Copyright Massachusetts Medical Society).

An optimal cut-off to discriminate men who are harbouring the disease from those who do not has not been found. It has been shown that the proportion of men diagnosed with a prostate cancer with a PSA of 2-3 ng/mL is approximately the same as the proportion of men who have a PSA higher than the normally used cut-off of 3 ng/mL [26]. The prostate cancer prevention trial showed that the risk of prostate cancer increases as PSA increases [27]. It is also clear from this trial that a large proportion men with an elevated PSA up to >6 ng/mL do

* In this article the authors used the Whitmore-Jewett staging, which is not commonly used today, where A1 disease represents the most highly differentiated cancer that has not spread and D when the disease has spread to other organs.

not harbour a prostate cancer, out of those 150 with a PSA >6 ng/mL 43.3% had a prostate cancer.

Diagnostic or surgical events such as biopsy of the prostate, TUR-P or TUR-B, also transiently increase the level of PSA in the blood [28]. The intra-individual change can also be up to 15-20% when PSA is analysed on different occasions [29,30]. DRE does not influence the level of PSA to such an extent that it plays a clinical role [31]. Normal physical activity does not raise the PSA although extreme cycling might raise the level if the blood sample is taken within a short time of the exercise, but there are other published papers with contradicting results [32-34]. Urinary tract infections cause an increase in PSA and the elevated value may be persistent for a very long time.

2.3.1.2 Free to Total Ratio of PSA

In the early 1990s it was discovered that not all PSA in serum binds to proteins, some also exists in its free form [35,36]. It was shown that the level of the bound vs. the unbound PSA differed among men with and without prostate cancer [36,37], and that a low free to total ratio was associated with a risk of prostate cancer [38-44]. In 1998, Catalona et al. published a paper in which they sought to address this issue and suggested to use a cut-off of 0.25 where a higher value indicated that, to a higher extent, BPH was responsible for the increase in PSA. The f/t PSA has been validated in PSA ranges of 4-10 ng/mL [45]. Swedish urologists have mainly used a cut-off of 0.18, which also has been validated for men with a PSA of 3 ng/mL or less. Men with PSA in these ranges and a f/t PSA >0.18 can probably be screened with longer intervals and do not have to undergo DRE as frequently as other men. But for men with a PSA below 3 ng/mL and a f/t PSA <0.18 with a normal prostate as defined by DRE, 9 out of 42 (21%) men undergoing a prostate biopsy harboured a prostate cancer [46]. As with PSA, no clear-cut line can be drawn for when to consider a value pathological or not. The free to total ratio is also affected by urinary tract infections and a low value can persist for a long time after the symptoms have passed [47].

2.3.1.3 Human Kallikrein 2

This protease shares approximately 80% amino acid homology with PSA, and it has been shown that human kallikrein 2 (hK2) activates pPSA to active PSA [48]. hK2 is measured in blood. It is expressed in higher levels in cancerous tissue than PSA and immunohistochemically it stains the tissue differently compared with PSA. More intense staining is seen in Gleason 4 to 5 compared with PSA [49,50]. hK2 is measured in much lower concentrations in the blood compared with PSA [51]. The additive value of using hK2

in combination with PSA is not clear, but might play a role to increase the specificity of prostate cancer testing in the low ranges of PSA [52]. The test has not yet been implemented in clinical routine in Sweden.

2.3.1.4 Single Nucleotide Polymorphisms – SNPs

Deoxyribonucleic Acid (DNA) is the chemical structure that holds the genetic information – it is organised in chromosomes. The human genome consists of 46 chromosomes where 23 are inherited from the mother and 23 from the father. A gene is a specific sequence of DNA that can be transcribed to a specific order of amino acids that constructs a protein. Each group of three nucleotides translates into a specific amino acid and the order of the amino acids is important for the function of the protein. Each nucleotide is called a base pair. Alterations in the genomic sequence and base pair can lead to alterations in the function of the protein, which can lead to either a disease or risk of developing a disease. These alterations are called single nucleotide polymorphisms.

A genome-wide association study is a classical case-control study where men with the trait are compared to men without it. Blood is generally the source of the DNA that is analysed. Usually a SNP array is used where millions of different SNPs can be analysed at the same time. If one variant is more common in men with the disease that variant is said to be associated with the disease. By using this method, the whole genome can be explored efficiently. In these types of analysis where more than a million SNPs are investigated in huge populations there will be false positive associations. By setting a "genome wide association significance" level (p) at $5x10^{-8}$ the large majority of the false positive results can be disregarded. Once a set of significantly associated risk SNPs has been identified they must be validated in at least one independent population to be truly confirmed as associated with the disease. The common way of reporting the association with a disease is to report the per allele odds ratio of the SNP. The median OR for SNPs associated with a disease is 1.3 and, very rarely, the OR is greater than 3. The variations in SNPs explain only a small part of one patient's risk of developing a certain disease.

An argument against these types of studies is that they are not hypothesis driven; they are more or less like a fishing expedition, where findings are interpreted afterwards. Many of the SNPs associated with a specific disease lie outside of exons in known genes, most likely in regulatory regions of the genome – regions whose functions are more or less unknown today. Although some of the SNPs associated with prostate cancer lie in known genes.

The first SNP shown to be associated with prostate cancer was described in 2006. It was located in the 8q24 region, which is intergenic [53]. It is thought that this region regulates the Myc expression, which is a known proto-oncogene [54]. It has been estimated that the heritability of prostate cancer is 58% [55]. The heritability is explained as what proportion of observed differences in a certain trait in a population that is explained by genetic variance. In the field of prostate cancer, 100 base pair alterations (SNPs) have been identified and confirmed to be associated with a risk of developing prostate cancer. These 100 SNPs are estimated to explain 33% of the heritability of prostate cancer. The most recent SNPs were published during the autumn of 2014 [56]. Zheng et al showed that men with a higher number of risk alleles in combination with family history had a higher risk of prostate cancer [57]. There is no clear evidence that any SNP is associated with the risk of developing an aggressive prostate cancer. One advantage of SNPs is that they only have to be analysed once – they do not change with age, prostate volume or infection. Since SNP analysis is cheap, almost the same cost as analysing a PSA, it is a marker that may be used in screening for prostate cancer.

2.3.1.5 PCA3

RNA-based tests are the most developed type of markers for prostate cancer in urine. In 1999 it was shown that prostate cancer cells express far more mRNA from the PCA3 gene than normal tissue and benign prostatic hyperplasia tissue. This mRNA does not produce any known protein so the level of mRNA has to be measured directly. Prostate cancer cells express in median 66 times more PCA mRNA than normal and hyperplastic tissue. After DRE the level of PCA3 mRNA can be analysed in the voided urine, however the PCA3 mRNA levels have to be normalised to the levels of PSA mRNA. Multiplying the ratio of PCA3 mRNA to PSA mRNA by 1000 then creates the PCA3 score. A Dutch multicentre study demonstrated that the PCA3 test had the same sensitivity but better specificity than PSA alone when testing for prostate cancer [58]. A TMA-based platform has been developed which is used after prostatic massage in normal voided urine. The test has been proven to work in a first biopsy setting where it improves the AUC when combined with PSA, prostate volume and DRE findings [59]. Although PCA3 may be used in the primary setting of identifying men who have a high risk of prostate cancer it is still too expensive to be used in everyday practice. PCA3 may play a role when deciding upon a rebiopsy when the initial prostate biopsy was negative for cancer in order to avoid a follow-up prostate biopsy [60]. This test is not routinely used in Sweden but there is a commercial test available.

Urine would make a useful fluid for prostate cancer screening, however the tests developed so far require prostatic massage before evaluation, which makes it unsuitable as a screening test today. The high cost of the commercial test lowers its utility as a screening test.

2.4 PATHOLOGICAL EVALUATION OF PROSTATE SPECIMENS

In order to diagnose a prostate cancer a microscopic evaluation of the tissue has to be made. A tissue examination of the prostate can reveal prognostic information. There are different methods to acquire the tissue. Either it is collected during surgery for other reasons – for example TUR-P, or it is decided that a man undergoing investigation for voiding problems or a suspicion of prostate cancer, should undergo a tissue sample. The tissue can be retrieved either by a FNA or a set of core biopsies of the prostate. The pathologist interprets the characteristics of the prostate cells and/or the gland structure to decide whether or not a prostate cancer is present. The architecture of the cells or glands is related to the prognosis of the disease. For tissue retrieved by FNA the grading is from 1 to 3, where 3 represents the least differentiated type [61]. In core biopsies, the Gleason score is reported. In some instances a clinical diagnosis of prostate cancer may be done without the need of specimen evaluation – for example when a man has an extremely elevated PSA, a palpable tumour and bone metastasis – then a clinical diagnosis can be made.

2.4.1.1 Gleason Grading System

A score is created based on the glandular differentiation and growth pattern of the prostate. The lowest score, 1, is attributed to glands with high differentiation most resembling the normal prostate tissue. Areas with lower differentiation are given a higher score, where 5 is the highest possible [62].

A Gleason score lower than 3 should rarely be used and almost never when grading prostate cancer in prostate biopsies. The Gleason sum, constructed by adding the score of the most prevalent pattern to the second most prevalent pattern, is reported by the pathologist. In 2005 an international consensus meeting was held and a modified Gleason grading was presented (Figure 5). The most important change was that the most aggressive area should always be reported. For example, a prostate tumour with a large area of Gleason 3 and a smaller area of Gleason 4 and a minute representation of Gleason 5 should now be reported 3+5=8 instead of 3+4=7 as it was reported in the initial grading system [63].

The Gleason grade is closely correlated to the prognosis after radical treatment as well as to the natural course of the disease [64-66].

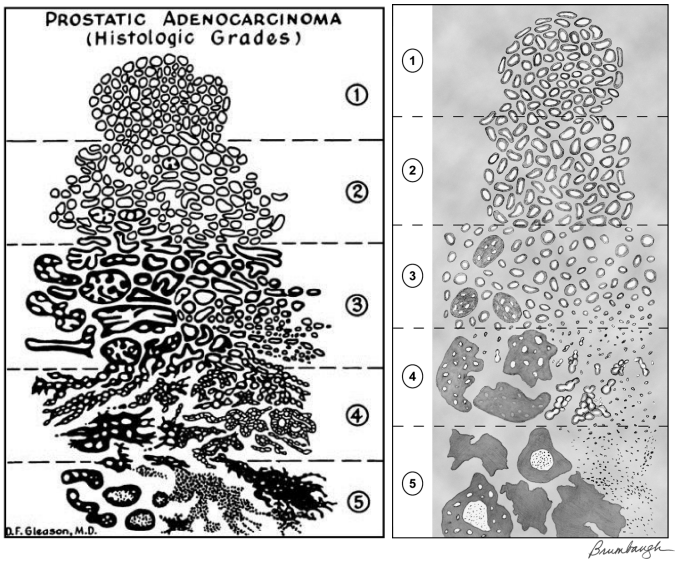

Figure 5.
On the left, the original Gleason pattern (by D.F. Gleason in 1966). On the right, the updated Gleason grading from the ISUP conference in 2005 [63] (Reprinted with permission, copyright).

2.5 CORE BIOPSIES OF THE PROSTATE

For histological evaluation of a specimen a larger chunk of tissue is needed than when doing a fine needle aspiration (cytological examination). By using core biopsies this can be achieved. This enables the pathologist to decide whether or not prostate cancer is present and to assign a Gleason Grade to a cancer.

During the late 1980s a sextant scheme was used, meaning that six cores were taken from the prostate. The needle is directed by using an ultrasound probe inserted to the rectum and biopsies are taken from the base, middle and the apical region of the prostate bilaterally. Modifications have been done and more cores have been added to the scheme. As of today, most urologists use a 10-12-core scheme directing the biopsies laterally as the cancer is more common in the peripheral zone of the prostate [67-69]. In repeat biopsies, performed when the initial round of biopsies were inconclusive or where there is still a suspicion of prostate cancer, some of the cores should be directed to the apical ventral and transitional zone of the prostate [70,71].

2.5.1.1 Taking prostate biopsies using image guidance

Traditionally, an ultrasound device has been used to guide the biopsy needle. The ultrasound probe is placed in the rectum together with a needle guide. The ultrasound aids the physician to direct the needle in the areas of the prostate that need to be sampled. The ultrasound does not aid the clinician to decide whether or not a cancer is present, but it assists in assessing the size of the gland and to introduce local anaesthesia before taking the biopsies.

Over the last few years MRI has won ground and the images produced can be fused into an ultrasound device thereby aiding the clinician to guide the biopsy needle to areas where there is a high suspicion of prostate cancer – so called fusion targeted biopsies. The MRI can also aid the clinician to direct the needle, without fusion technology, to a suspect lesion possibly reducing the number of unnecessary penetrations of the rectal mucosa [72]. In a repeat biopsy setting, the MRI is good at detecting anterior prostate tumours that are missed in the regular primary biopsy scheme [73,74]. A recent publication shows promising results when including the MRI in the initial evaluation of a patient with a suspicion of prostate cancer to avoid diagnosing men with low-risk tumours and to reduce the number of cores needed to find clinically significant prostate tumours [75]. But there is still not enough evidence or health economical benefits to include MRI in the standard evaluation of men with a suspicion of prostate cancer [76].

2.6 COMPLICATIONS FOLLOWING A PROSTATE BIOPSY

Prostate biopsies are usually done as an outpatient procedure. The patient is recommended prophylactic antibiotics before the procedure. The probe is inserted in the rectum following a palpation of the prostate. Local anaesthesia is administered to minimize the discomfort. The needle goes through a channel in the rectal probe and then a guidance line shows where the needle will take the tissue sample. The majority of patients tolerate the procedure but find it somewhat uncomfortable. Regular anaesthesia is rarely needed. A summary of the most common complications is presented in table 1.

Table 1. Complications following a prostate core biopsy. A summary of several studies [77-89]

Symptom	% affected men after biopsy
Haematuria	33.8 - 64.5
Haematospermia	6.0 - 90.1
Rectal bleeding	11.5 - 40.0
Acute urinary retention	0.11 - 1.7
Urinary tract infection	0.9 - 6.0
Bloodstream infection	0 - 2.8
Hospitalisation	0 - 6.9

2.6.1.1 Pain

In early years the transrectal core biopsies were performed without local anaesthesia [90,91]. This was to some extent acceptable when only a few cores were taken. As a consequence of the development of more extensive biopsy schemes it has been shown that local anaesthesia is effective in reducing the pain experienced by the patient [92]. The European Guidelines for Prostate Cancer consider local anaesthesia as "state of the art" [76].

2.6.1.2 Bleeding

Rectal bleeding is experienced by almost half of the men undergoing a prostate biopsy. This usually fades away within 12-24 hours. Haematuria (blood in the urine) is also common and usually diminishes within a few days. Haematospermia (residual blood in the ejaculate) is very common – most patients experience it and notice it for up to four weeks although some may complain of it for up to a couple of months afterwards. This is not dangerous for the patient or his partner. Bleeding complications rarely require hospital treatment [93].

2.6.1.3 Infection

Infectious complications vary from mild urinary tract infections to severe septic shock and, in rare cases, death. Up to 6% of men undergoing a prostate biopsy experience mild fever and have a positive urine culture after the procedure. A recent Swedish study claimed that 6% of patients undergoing a prostate biopsy receive a prescription of a urinary tract infection related antibiotic within 30 days of the procedure [84]. Up to 2.8% have a sepsis after the procedure [94]. In comparison to other European countries, Sweden has been relatively spared from the problem of multi-resistant bacteria. One of the theories behind this is that Swedish health authorities and doctors are cautious in prescribing antibiotics. The antibiotic use per capita in Sweden is among the lowest in Europe [95-98]. Over the last few years, however, a significant increase in the number of infections caused by these resistant strains of bacteria has been noticed internationally, including in Sweden [99,100].

2.6.1.4 Clinical routine in Sweden with regards to prophylactic antibiotics

All men are recommended prophylactic antibiotics before the procedure [101]. The standard regimen used in Stockholm, Sweden, is ciprofloxacin 750 mg before the procedure. For men allergic to ciprofloxacin or where risk factors are present other regimens are used. To this date, rectal swabbing and microbial cultures preceding the prostate biopsy are not routinely used in Sweden, unless there is a suspicion of an on-going UTI. If there is an on-going UTI the biopsy should be postponed.

2.6.1.5 Mortality rates after prostate biopsy

Although a prophylactic antibiotic is used and a careful history is taken with regards to bleeding disorders or history of medications prolonging the bleeding time, patients do experience serious adverse events [93]. Published studies reporting mortality after a prostate biopsy show contradictory results. The ERSPC trial published data from three centres where no excess mortality at 120 days following biopsy could be seen. The mortality rate in this study was 0.24% for men undergoing a prostate biopsy as well as for men in the control group [102]. In the PLCO trial the 120-day mortality rate following a prostate biopsy was 0.95/1000 compared to 1.8/1000 in the control arm [103]. In a Canadian population, the 120-day mortality rate was 1.3% whereas in a control population it was 0.3% [104]. The difference in the studies is still present, but slightly smaller, when restricting the analysis by age from PLCO to match the Canadian study. Another reason might be that the compliance to biopsy in the PLCO trial was low and that a "healthy volunteer" bias was introduced, that is that the men ending up undergoing a prostate biopsy are those who are most concerned about their health and are thus more healthy and not as receptive to complications.

2.7 STAGING OF PROSTATE CANCER

The most widely used system for the classification of prostate tumours is the TNM system. It is based on the growth of the tumour, whether or not it is confined to the prostate, and its relation to the capsule and nearby structures (Table 2). The "N" and "M" refers to the presence of lymph node and bone metastasis, respectively. To evaluate if lymph nodes are affected by prostate cancer, a pelvic MRI is done. Bone metastasis is evaluated by bone scintigram where a radioactive isotope is injected in the blood stream. The isotope is enriched in areas of high skeletal metabolism such as fractures and bone metastasis. This information is used to describe the disease and to guide the clinician in suggesting the right treatment for the patient.

Table 2. TNM classification of prostate cancer [105].

T	**Primary Tumour**		
	TX		Primary tumour cannot be assessed
	T0		No evidence of primary tumour
	T1		Clinically inapparent tumour neither palpable nor visible by imaging
		T1a	Tumour incidental histologic finding in 5% of less of tissue resected
		T1b	Tumour incidental histologic finding in more than 5% of tissue resected
		T1c	Tumour identified by needle biopsy (eg. because of elevated PSA)
	T2		Tumour confined within the prostate
		T2a	Tumor involves one-half of one lobe or less
		T2b	Tumour involves more than one-half of one lobe but not both lobes
		T2c	Tumour involves both lobes
	T3		Tumour extends through the prostatic capsule
		T3a	Extracapsular extension (uni- or bilateral)
		T3b	Tumour invades the seminal vesicle(s)
	T4		Tumour is fixed or invades adjacent structures other than seminal vesicles: bladder, levator muscles and/or pelvic wall
N	**Regional Lymph Nodes**		
	NX		Regional lymph nodes were not assessed
	N0		No regional lymph node metastasis
	N1		Metastasis in regional lymph nodes
M	**Distant Metastasis**		
	M0		No distant metastasis
	M1		Distant metastasis
		M1a	Non-regional lymph node(s)
		M1b	Bone(s)
		M1c	Other site(s) with or without bone disease

2.8 RISK STRATIFICATION OF PROSTATE CANCER

Depending on the PSA level and characteristics of the tumour, men are stratified into different risk groups. Depending on the risk group different treatment options are available. D'Amico developed the most widely used stratification tool in the 1990s. Stratifying men into risk groups depending on Gleason Grade, PSA and palpatory findings can be a helpful tool when deciding upon relevant treatment for the individual patient [106]. The Swedish national health group for prostate cancer has introduced a new group (very low risk) for men with a very low risk of progression (Table 3).

Table 3. Risk group stratification used by the Swedish National Health Group for Prostate Cancer, which is based on the D'Amico criteria.

Very low risk	T1c, sum ≤6, 1-4 cores positive for cancer out of a total of 8-12 cores, ≤8 mm of total cancer length and a PSA density < 0,15 μg/l/cm^3
Low risk	T1a-T2a, Gleason sum ≤6 and PSA ≤ 10ng/mL
Intermediate risk	T2b and/or Gleason sum 7 and/or PSA 10-19.9 ng/mL
High risk	T2c-T3 and/or Gleason sum ≥ 8 (or extensive growth of 4+3 in more than 50% of the cores taken) and/or PSA≥ 20ng/mL

2.8.1.1 Prognosis of prostate cancer based on risk group stratification

The cumulative proportion of men who died due to prostate cancer where no curative treatment was performed at the time of diagnosis was associated with the stage of the tumour at the diagnosis in a Swedish study. The 10-year cumulative proportion of men who succumbed due to prostate cancer was 4.5, 13, and 29% in the low, intermediate, and high-risk group, respectively. The 15-year cumulative proportions in the corresponding risk groups were 9, 20, and 36% respectively [107]. In an American study, the same pattern was seen in a study where no curative treatment was given. Men with a high Gleason score, 8-10, had a mortality rate of 121 deaths/1000 person-years whereas men with a Gleason score of 6 had a mortality rate of 30 deaths/ 1000 person-years [108].

2.9 SCREENING FOR PROSTATE CANCER

To screen a population for a certain disease has been, and still is, a controversial subject. When screening is introduced, the incidence rate of the disease increases and there is usually a stage shift of the disease – that is the disease is discovered at an earlier stage because a larger proportion of the diagnosed tumours will be detected earlier. The benefits of an earlier diagnosis are that a larger proportion of the diagnosed patients could be offered curative treatment and thereby symptoms and possibly the risk of dying due to the disease are reduced. The pool of prevalent cases will also increase with an increase in the number of controls of the patients that have to be undertaken. One objection against screening is that it detects a disease in individuals who will never go on to develop symptoms of the disease. They will suffer the consequences of the treatment but not benefit from them. This is especially relevant in prostate cancer where there is a long lead-time from detection to symptoms of the disease.

The most obvious outcome for screening is to reduce mortality for a specific disease. How many men have to be screened to avoid one death is described by the number needed to screen. In screening studies this is done by calculating the absolute risk difference of death in the two arms of the study and then inverting this number. This number will indicate how effective the screening is in terms of how many men must be invited and participate in the screening in order to prevent/avoid one death due to the specific disease.

2.9.1.1 European Randomized Screening Trial for Prostate Cancer

ERSPC is a multicentre trial evaluating PSA as a screening tool for prostate cancer with death from the disease as the primary end-point. Seven European countries were involved and 72 890 men in the core ages 55-69 years were included in the screening group and 89 535 men were included in the control group. 82% of the screening group were screened at least once. The different countries had slightly different inclusion and follow-up criteria. Most centres used a cut-off of 4 ng/mL to recommend a prostate biopsy. The screening group had a reduced rate of prostate cancer related death of 20% compared with the control group at a median follow up time of nine years. The absolute risk difference of dying from prostate cancer was 0.00071, which translates to 1408 men having to be screened in order to avoid one death from prostate cancer. A follow-up published three years later showed that the absolute risk difference of dying from prostate cancer between those screened and those not increased with time. It was estimated that the absolute risk difference was 1.07 deaths per 1000 person years, corresponding to that 935 men were needed to be screened to avoid one death in prostate cancer. A follow up of the Swedish part of the ERSPC, conducted in Gothenburg, showed that the number of men needed to be screened to avoid one death in prostate cancer had declined to 293 after a median follow up time of 14 years. The number of men needed to be diagnosed was at this time point 12. It is, however, important to remember that a large proportion of the men who are diagnosed with prostate cancer actually have a prostate cancer which most likely will not affect their life expectancy [9,109-111].

2.9.1.2 Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

From 1993 to the end of 2001, men and women between 55 and 74 years of age were recruited to participate in the study at 10 centres in the United States. Primary exclusion criteria were: current cancer treatment, history of any of the investigated diseases and, from 1995 onwards, more than one PSA test in the preceding three years. The male screening group (n=38 343) was offered annual PSA testing for six years and DRE annually for four

years. Men with pathological findings were recommended to seek diagnostic evaluation from their primary physicians. The primary end-point was cause-specific mortality.

The PLCO trial did not show any positive effect on prostate cancer mortality after 10 years. This lead to the recommendation, by the U.S. Preventive Services Task Force, not to recommend screening for prostate cancer in the United States [112]. However, concerns have been raised regarding the fact that 40 to 52% of the men in the control group had their PSA analysed, which might have reduced the differences between the two study-arms thereby introducing a false result. A second argument as to why this study did not show any benefits of screening was that only 41% of the men with a first positive screen test underwent a prostate biopsy within one year and 64% within three years. For men with a negative PSA test but a positive DRE only 27% underwent a prostate biopsy within three years [113].

2.9.1.3 Opportunistic PSA testing

No country has so far established a screening programme for prostate cancer. But in some countries the frequency of PSA testing is so common it may be called opportunistic screening. In Stockholm, Sweden, more than 60% of men older than 60 years of age have had their PSA analysed in the last five years, and more than 50% of them have been retested within 26 months, irrespective of their initial PSA [114]. The opportunistic screening seems to have some effect on prostate cancer mortality, but the results are not as good as the results reached in structured trials. It has been shown that in areas where PSA testing is common, such as Stockholm, the mortality rate is lower than in areas where testing is not as common. The rate ratio for prostate cancer mortality in counties with a high degree of PSA testing compared to counties with low degree of testing has been described to be 0.81 [115]. This suggests that for PSA testing to have a substantial effect on prostate cancer mortality rates the testing and follow-up must be structured.

2.10 TREATMENT OF PROSTATE CANCER

2.10.1.1 Radical Treatment

The first question the treating physician has to ask is if the patient will benefit from a radical treatment of his condition. For men with a low-risk tumour, the risk of dying of prostate cancer is small and these men should rarely be recommended radical treatment – especially if they are older than 65 years. Men younger than 65 years of age and men with an intermediate risk prostate cancer seem to benefit the most from radical treatment. However, there is a

reduction in risk of distant metastasis for men in the older age groups, which could be an argument to perform surgery on men older than 65 years of age [108,116].

The assumption that men older than 65 years of age do not benefit from radical treatment is based on studies that included patients in the late 1980s or early 1990s, when the median age of death in men was lower than it is today. Urologists have adapted to this and now the question that is asked is whether or not the man has more than 10 years life expectancy - looking more at biological rather than chronological age. If the life expectancy is estimated to be more than 10 years the patient will most likely benefit from radical treatment. Information on comorbidities can guide the clinician whether or not to recommend the patient to undergo radical treatment. A study based on the NPCR estimated the survival rates for men with different stages of prostate cancer stratified on their CCI. Men with a high CCI had worse overall survival than men without any comorbidity and a greater risk of dying from other causes than prostate cancer [117]. This suggests that the choice of treatment should be based on the patient's medical history. Older men with a life expectancy larger than ten years with a high-grade disease are likely to benefit from radical treatment and should be offered radical treatment, although they have to be informed that there is a substantial risk of side effects in these ages. The numbers needed to treat to avoid one death from prostate cancer have been shown to be 8 for surgical intervention at 18 years and 10 for radiation therapy in combination with hormonal treatment at 10 years [116,118].

The most common negative consequences of radical treatment, for both radiation therapy and radical prostatectomy, include the risk of incontinence and impotence[119-122]. For radiation there is also a risk of suffering from inflammation of the rectum. The impact of the side effects has been one of the reasons why prostate cancer screening has not been implemented.

2.10.1.2 Active Surveillance

Prostate cancer is a disease with a very broad span of prognosis. A large proportion of the diagnosed tumours have a very low level of activity and seem to cause no harm to the men whereas some tumours are aggressive and most certainly alter the life span of a diagnosed man. Several studies have shown that the numbers needed to treat to save one man from death from prostate cancer are relatively high – meaning that a lot of men will undergo the procedures and suffer the consequences but not benefit from the treatment [116,123]. One large American study has shown that men with a low-risk disease can survive for a long time without developing metastasis or dying from the disease [124].

Swedish health authorities recommend active surveillance for men with a low-risk disease, although the patient's opinion is to be taken into account when making the decision [101]. This treatment alternative has increased during the last years. In Sweden the proportion of men with a very low risk prostate cancer, who has been offered active surveillance as their primary treatment, has increased from 55% in 2009 to 85% in 2013 [125].

2.10.1.3 Non-curative Treatment of Prostate Cancer

Men who are diagnosed with prostate cancer and have a life expectancy shorter than 10 years and/or a low grade localised disease may be recommended watchful waiting or deferred treatment [76]. This indicates that no treatment is initiated until the patient develops symptoms from his disease. This may include voiding problems or pain from skeletal metastases. When a tumour has progressed this far it is usually not curable but good palliative care, such as hormonal treatment, is efficient in reducing symptoms. Hormonal treatment strives to reduce testosterone levels since prostate cancer cells are testosterone-dependent. Either a surgical or medical castration is recommended to decrease the testosterone levels in the body. If the side effects of castration are unwanted or if the tumour is locally advanced an anti-androgen therapy, which inhibits the uptake of testosterone in the cells, may be used.

2.11 EPIDEMIOLOGICAL RESOURCES AND SWEDISH REGISTERS COMMONLY USED IN PROSTATE CANCER RESEARCH

2.11.1.1 The Swedish Personal Identification Number

This code, which is based on the date and year a person is born plus four additional digits including a control digit, is what gives strength to Swedish registers and epidemiological research. The PIN is unique for each citizen and was primarily introduced to keep track of the population for tax and military purposes. It has been in use since 1947 with only minor adjustments. All registers use the PIN as the personal identifier and thereby it is possible to link registers to each other [126].

2.11.1.2 The National Prostate Cancer Register

The National Prostate Cancer Register started to collect clinical data on all men diagnosed with prostate cancer from 1998. Before, this information was collected on a regional basis but, through a joint effort, one register for the whole of Sweden was created. Information on TNM classification, PSA at diagnosis, and Gleason Grade is recorded. The register also contains information on primary treatment and, during the later years, the results of treatment.

The register and information collected has been updated on a few occasions. This register covers >98% of all the tumours in the Swedish Cancer register [127].

2.11.1.3 The Swedish Cancer Register

Since 1958, the Swedish Cancer Register has collected information on all the cancers diagnosed in Sweden. Both the pathology laboratories and the clinicians diagnosing a cancer are obliged by law to report diagnosed cancers to the register. The Swedish Cancer register contains information on WHO grade and TNM classification and how the tumour was diagnosed. Validation studies have been performed and the register covers more than 98% of all tumours diagnosed [2,128].

2.11.1.4 The National Patient Register

The national patient register started registering all hospitalisations for all people receiving health care in Sweden. The inpatient part started collecting data in 1964 and contains information on main and secondary diagnoses and surgical procedures done (based on ICD-10), if any, during the hospital stay and at which hospital the health care was given. The date of admission as well as the date of leave is registered. The outpatient part of the register does not contain information from primary care physicians [129].

2.11.1.5 The Swedish Cause of Death Register

The register used today was established in 1961. It uses the ICD codes and is updated annually. At every death a doctor has to report the primary cause and any underlying diseases that may have contributed to it for Swedish residents. Based on international standards an illness is set as primary cause of death alongside contributing diagnoses. The register is complete for up to 99% of all people registered in Sweden regardless if the death occurred in the country or elsewhere in the world [130].

2.11.1.6 Total Population Register

Sweden has had a long history of keeping track of its inhabitants. This was done by the local churches, which reported to the state for tax and military purposes. The Swedish Tax agency took over the responsibility in 1991 and provides information on each citizen´s PIN, sex, birth, address, marital status, and country of birth, emigration and immigration as well as date of death. This resource is useful for population-based research when person-time has to be taken into account.

2.12 STHLM COHORTS

2.12.1.1 STHLM-0

STHLM-0 is a register-based cohort consisting of men who have done at least one PSA test in Stockholm County since 2003. Data have been retrieved from the three laboratories, Karolinska Universitetslaboratoriet (KUL), Aleris Medilab (AM), Unilabs (UL), doing all PSA analysis in Stockholm, including information on total and free PSA where available as well as date of analysis.

The men were linked to the pathology registers where information on date of incoming prostate samples and result of the pathology report were retrieved. These data are updated 2-3 times per year. The data has been linked to the National Prostate Cancer Register, Swedish Cancer Register, National Patient Register, Swedish Prescription Register, Swedish Cause of Death Register, Educational Register and Total Population Register. The men who have undergone a prostate biopsy have also been linked to the Swedish Intensive Care register and to the microbiological databases at the departments performing all blood cultures in Stockholm. As of 1st of January 2014 this database contain information on 410 000 men with at least one PSA test in the Stockholm area.

There is missing data with regards to free PSA which is because the results of the analysis performed by the laboratory were dictated to the analysis demanded by the physician ordering the blood sample and only ordered analyses have been saved. There is also incomplete data regarding PSA analysis for the southern part of Stockholm from 2003 to the beginning of 2006 due to loss of data from the laboratory. This represents only 15% of the PSAs analysed during this time period.

2.12.1.2 STHLM-1

Men who between 01/012005 and 31/12/2007 underwent a prostate biopsy were invited to donate blood for SNP analysis. Exclusion criteria were: age above 80 years, other cancer than prostate cancer diagnosed at the prostate biopsy, prior prostate cancer diagnosis, deceased at the time of invitation or a non-valid Swedish personal identification number. 7035 men were invited and 5241 accepted participation, donated blood, and filled-out a questionnaire covering family history of prostate cancer. The men were linked to the PSA registers and NPCR. 2135 of them had a first prostate biopsy positive for cancer regardless of PSA level. 3791 men had a PSA <10 ng/mL, out of which 1359 were diagnosed with a prostate cancer.

2.12.1.3 STHLM-2

During 2010 and 2011 men who for any reason were recommended to analyse their PSA were invited to participate in the STHLM-2 cohort at the time of the blood sample procedure. PSA was analysed by ordinary methods, extra blood and urine samples were collected and the men were asked to fill out a web-based questionnaire. A total of 27 350 men were included and linked to the above-mentioned registers by using the STHLM-0 cohort. The cohort consists of men without prostate cancer, men with an already known prostate cancer, and men who, within a relatively short time after the PSA test, were diagnosed with prostate cancer. This cohort has been used for validation studies of the biomarker panel used in the STHLM-3 trial.

3 AIMS OF THE THESIS

The overall aim of this thesis was to investigate prostate biopsy patterns and trends and to explore and improve prostate cancer diagnostics as well as describing the increasing problems with multi-resistant bacteria and its implications for the diagnosis of prostate cancer

In particular, the thesis aimed:

- To evaluate if the single nucleotide polymorphisms associated with prostate cancer can be used to reduce the number of unnecessary prostate biopsies for men with a PSA of <10 ng/mL
- To evaluate if the single nucleotide polymorphisms associated with prostate cancer can be used to identify men with a higher risk of prostate cancer in men with PSA 1-3 ng/mL
- To describe prostate biopsy patterns and time from PSA test to prostate biopsy in men living in Stockholm, Sweden
- To evaluate if serious infectious complications after prostate biopsies are increasing in Stockholm, Sweden

4 MATERIALS AND METHODS

4.1 STUDY POPULATIONS

4.1.1.1 Study I

A total of 8088 men who had undergone at least one prostate biopsy were identified by retrieving data from two out of three pathological departments in Stockholm between 1 January 2005 and 31 December 2007. These two departments analyse approximately 75% of all prostate biopsies done in the region. Exclusion criteria were: age above 80 years, no valid PIN, deceased at the time of invitation, a prostate cancer diagnosis prior to 1 January 2005 and other cancer than prostate cancer diagnosed at the prostate biopsy. In total, 7035 men were invited and 5241 accepted and donated blood samples. These men were linked to laboratory databases to match PSA values to their prostate biopsies. For cancer status and clinical information regarding the tumours the men were linked to the NPCR and the SCR. The analysis was further restricted to men with a PSA lower than 10 ng/mL – the reasoning behind this selection is that in this PSA range only 25% of men will have a positive biopsy and hence a greater specificity would save a large proportion of the unnecessary biopsies. SNP selection was based on all published PC-associated SNPs until October 2009. The SNPs used had to be validated in at least one independent study population. Genotyping was done using MALDI-TOF mass spectrometry based on allele-specific primer extension with iPLEX chemistry (Sequnom Inc., San Diego, CA, USA). Hardy-Weinberg calculations were performed. A total of 36 SNPs were genotyped in the entire sample, rs2660753 failed completely, and the others had an average success rate of 98.6%.

The genetic risk score was created by summing the number of risk alleles at each of the 35 successful SNPs multiplied by the logarithm of that SNP's OR. Aggressive cancer was defined as T3-T4, N1, M1 or Gleason 4+3 or higher.

4.1.1.2 Study II

From the STHLM-2 cohort, men with a PSA between 1 and 3 ng/mL and between 50 and 69 years of age at the time of inclusion were identified. 2696 men with no prior history of prostate cancer or prostate biopsies were identified and genotyped. For the genotyping, we selected all known SNPs reported to be associated with prostate cancer and replicated in at least one independent study population and published before October 2012. In total, 50 relevant SNPs were chosen. The ORs and risk allele frequency were calculated by using a nested case control population from the STHLM-2 cohort. We used the same genotyping

method as in Study I and the average success rate was 98.7%, rs13385191 failed completely. The score was created in the same way as in Study I. Based on the genetic risk score a man was categorised as belonging to the low, intermediate or high-risk group.

A randomised stratified selection (based on their genetic score) of men was invited to participate. Based on the power calculation it was estimated that 200 men needed to undergo a prostate biopsy. From earlier studies we knew that the response rate would be high and therefore a total of 860 men were invited. There were 200 time slots available for prostate biopsies and 192 men signed up for a prostate biopsy, 14 were not medically fit or were using anticoagulative medication, 6 men did not show up at the scheduled appointment. Two urologists, who were blinded to the genetic risk score, performed the biopsies in the 172 men. The prostate biopsies were taken using a pre-set scheme with 10 laterally directed cores if the gland was smaller than 35cc and 12 cores if it was larger. An ultrasound-guided technique was used, and all patients received prophylactic antibiotics and local anaesthesia. One pathologist, who was also blinded to the genetic risk score, evaluated all specimens.

4.1.1.3 Study III

From STHLM-0 we identified all prostate samples between 1 January 2003 and 31 December 2012 (no. of specimens =56 014). The men and the dates of their prostate biopsies (data from KUL, AM and UL) were linked to the NPR to exclude specimens taken during other surgical interventions such as TUR-P and TUR-P (no. of specimens excluded=11 536). The men were also linked to the TPR in order to adjust for emigration outside the Stockholm area (no. of specimens excluded = 2193). When restricting to the years studied, 2004-2012, there were 38 880 prostate biopsies performed during this time period. In a subgroup analysis we selected men who at the time of biopsy were diagnosed with an advanced prostate cancer defined as ≥T3, N1, M1 or PSA ≥20 ng/mL at diagnosis. In total, 4 236 men met this definition and 1497 were aged 50-69 years and had no prostate biopsy recorded prior to their diagnostic prostate biopsy.

4.1.1.4 Study IV

From STHLM-0 we identified all men who had undergone at least one prostate biopsy from 1 January 2003 to 31 December 2012. These men were linked to NPR, NPCR, CR, SIR, and SCOD. The reasons for not linking these men to the TPR were: 1) as the outcome studied, having a positive blood culture, in most cases occurred within a week and a minute proportion of the men migrated in this time interval. 2) Data on hospital admission from the NPR and the SCOD is nation-based, which means that a man undergoing a prostate biopsy in

Stockholm and is admitted to a hospital or who died shortly after the biopsy elsewhere in the country will be registered. Data was retrieved from the clinical microbiological laboratories performing blood cultures (KUL and UL) and data concerning results and biograms were retrieved for those who were positive. In the cohort used for analysis we had access to 56 076 prostate specimens. When excluding histological specimens other than core biopsies of the prostate and restricting to age > 30 at time of biopsy there were 44 047 biopsies left performed in 32 196 men from 2003 to 2012.

4.2 STATISTICAL METHODS

All p-values were based on two-sided hypothesis and $p<0.05$ was considered statistically significant. All statistical analyses were performed using SAS 9.2, R or STATA 11.2 for Mac.

4.2.1.1 Statistical Models

4.2.1.1.1 Logistic regression

Logistic regression is used in epidemiological research when the relationship between a dichotomous variable and one or more independent variables is analysed. By adding the independent variables one at a time the effect of the variable and the effect of confounding can be appreciated. In a univariate analysis, only one independent variable is used to predict the outcome, whereas in multivariate analysis several independent variables may be added. Logistic regression can also be used to predict the probability of an event occurring. The odds are defined as the probability of an event divided by 1 minus the probability, and the odds ratio (OR) is calculated by dividing the odds for one group divided by the odds for another group and it is used to compare the probabilities of a certain event occurring for two different groups. In these studies the estimated OR is reported with 95% confidence intervals. Used in *Studies I, II, III, IV*.

4.2.1.1.2 The Kaplan-Meier estimator

The Kaplan Meier function can, for example, be used to estimate the proportion of men surviving a specific disease at a certain time after an exposure and can thus be used to compare the outcome of different treatments. The model is flexible and can be used to estimate any event dependent on time and allows for stratification on different groups. An event does not have to be a single event but can be a grouping of several events, as in study III. The function takes every individual's attributed time into account and is thereby useful

when study subjects are lost to follow-up, die, or do not want to be part of the investigation any longer; the study subject is in other words censored. The proportion of survivors is calculated at certain time points where the censoring is taken into account – thereby adjusting the calculated proportions to the maximum of retained study subjects and in the next set of calculations the number of men at risk is the total number of men left in the life table.

In our studies we frequently plotted the result as 1- survival rather than the survival to illustrate the cumulative effect of the event. Used in *Study III.*

4.2.1.1.3 Net reclassification index

This test is used to measure the improvement of a prediction model when adding a variable. It was presented by Pencina in 2008 and is used when new biomarkers are tested [131]. It was developed to complement the AUC (the area under the receiver operating characteristics curve; a plot of sensitivity vs. 1-specificity over a wide range of cut-offs for a specific biomarker), as this may be cumbersome to interpret and because very strong associations between a new marker and the outcome of a model only lead to tiny changes in the AUC. The improvement or reclassification compares the subjects being correctly classified to the ones being wrongly classified in the two groups experiencing the event or not. A positive NRI indicates that the new biomarker increases the model's predictive performance. Used in *Studies I, II.*

4.2.1.1.4 Standard Mortality Ratio

The standard mortality ratio is used when a comparison wants to be made between the study subjects and the overall population. By dividing the observed deaths in the cohort with the expected number of deaths calculated by the age-standardised mortality rate a ratio is given. If the ratio is larger than 1 the studied group has an increased mortality compared with the other group. Care has to be taken when choosing the comparison group with regards to age and exposure of risk factors. The results may differ quite substantially and, thereby, the interpretation of the results. Used in *Study IV*.

4.2.1.2 Test of significance and trends

4.2.1.2.1 Cochran - Armitage test for trend

This test is used to test the presence of an association between one variable with two categories and a variable with several categories. It is a variant of the Pearson chi-squared test, which is used to establish whether or not an observed frequency of an event differs from

the expected frequency. It is most useful to confirm when there is a suspected trend in the data. It is commonly used in genetic association studies. Used in *Studies I, II, IV*.

4.2.1.2.2 Cuzick's test for trend

Cuzick's test is a development of the Wilcoxon rank-sum test in which a variable is tested for three or more ordered groups. It is a non-parametric test, which makes it robust against distribution assumptions. Used in *Study II.*

4.2.1.2.3 Wald's test

Wald's test is a parametric test used in logistic regression analysis to test the significance of separate coefficients. It is equal to the coefficient in the logistic regression divided by the standard error. The square of the test statistic is asymptotically chi-square distributed with one degree of freedom, a property that is applied to compute p-values. If the sample size is large Wald's test gives similar results as the likelihood ratio test. Used in Study IV.

4.2.1.2.4 Likelihood ratio test

The likelihood ratio test is used to test the goodness of fit between two models. A model with more variables is compared with a simpler model to see if it fits the observed data better. A more complex model will always be at least as good as the simpler model and, in most cases, it will be better and have a higher likelihood score. To obtain the test statistic, the difference in the logarithm of the likelihood score for the more complex model and the basic model is computed and then multiplied by two. This test statistic is asymptotically chi-squared distributed with degrees of freedom equal to the difference in number of parameters in the more complex model and the basic model. If the test is significant the more complex model is said to fit the data better compared with the basic model and, therefore, the added variables are of importance. Used in *Study I, II, IV*.

4.3 STATISTICAL ANALYSIS

4.3.1.1 Study I

The association between each SNP and prostate cancer at biopsy was tested using the Cochrane-Armitage test for trend. The OR with 95% CI for each SNP was calculated by using logistic regression. Age, family history, PSA and f/t PSA were the known risk factors and their association with prostate cancer at biopsy was explored using logistic regression.

When using the same dataset to both fit the prediction model and explore the model's predictive capacity, there is always a risk of overestimating the predictive performance of the included variables. Cross-validation can be used to mitigate this effect. In cross-validation,

the dataset is partitioned into training and prediction sets, where the model is fitted to the training set and then used to predict the outcome in the prediction set. This is repeated for as many times as the original dataset has been tiled. Each time a new part of the original data set is used for the prediction. At the end, the average result of the coefficients in the prediction model is reported. In this study we performed a 10-fold cross validation.

Three models were created and compared: 1) non-genetic model combining age, PSA, f/t PSA and family history; 2) same as 1 and adding the genetic risk score; 3) a hypothetical genetic prediction model. These three models were compared using a likelihood ratio test. Specificity was tested using the net reclassification index. The predictive performance was estimated by calculating the AUC for each model. All reported p-values were based on two-sided hypothesis.

The hypothetical genetic prediction model (optimal genetic) was created under the assumption that the sibling relative risk of developing a prostate cancer is 2.5 and that the model is polygenic. By retracting the calculations in Pharaoh et al. the predictive performance of such model can be estimated [132].

4.3.1.2 Study II

A logistic regression model based on PSA, f/t PSA, age, family history, DRE and prostate volume was created and compared with a logistic regression model with the same variables with the addition of the genetic score. The different models were compared using model fit by likelihood ratio testing. The predictive performances of the regression models were compared using the net reclassification index. Cuzick´s test for trend was used to compare the different risk strata and the findings of cancer in each of them.

4.3.1.3 Study III

4.3.1.3.1 Classifying prostate core biopsies

Biopsies prior to a cancer diagnosis were classified as either the first recorded diagnostic biopsy or a subsequent diagnostic biopsy. We further classified diagnostic biopsies as being either positive for cancer or not. The reference date of a biopsy associated with a prostate cancer was defined as either: (i) the date of cancer diagnosis for men with a prostate cancer diagnosis based on TUR-P, clinical diagnosis or cytology; (ii) the date of biopsy referral for biopsies within 30 days of a cancer diagnosis or, otherwise; (iii) the date of cancer diagnosis. All biopsies performed more than 30 days after the reference date were assumed to be active surveillance biopsies. For all analyses, we included biopsies performed during 2003, giving at least 12 months of biopsy history.

4.3.1.3.2 Statistical modelling

To estimate the proportion of men who had a biopsy or other clinical investigation as a function of time from a first PSA test, we considered an event as the earliest biopsy, a repeat PSA test that was lower than 4 ng/mL, or a cancer diagnosis. We censored men at emigration, end of study follow-up (31 December 2012) or death. We did not censor for a subsequent PSA test, as this is potentially informative for having a subsequent biopsy. The variance estimates for the Kaplan-Meier estimators were not adjusted for repeated events within the same individual and confidence intervals should be interpreted cautiously. As a sensitivity analysis, we calculated the Charlson Comorbidity Index (CCI) based on hospitalisations in the year preceding the PSA test and stratified time to subsequent biopsy or clinical investigation by a CCI of 0.1 or 2+. The CCI was calculated for ICD-10 using SAS code from the Manitoba Centre for Health Policy.

4.3.1.3.3 Subgroup analysis

We defined men with advanced prostate cancer as having a T3, T4, N1 M1 stage tumour or a PSA of ≥20 ng/mL at the time of diagnosis. We reported results for men aged 50-69 years, since such men were more likely to be candidates for radical treatment if they would have been diagnosed at a lower stage.

We investigated the proportion of men with advanced prostate cancer that had an earlier PSA test. For the time t prior to a cancer diagnosis we used the Kaplan-Meier estimator to calculate the cumulative proportion (=1-survival) of a PSA >4 ng/mL between time t and eight years prior to diagnosis, taking account of emigration.

4.3.1.4 Study IV

Frequencies and proportion biopsies that were followed by a blood culture within 30 days were calculated, as well as the proportion of positive cultures and bacteria findings.

Crude- and adjusted logistic regression models were used to estimate odds ratios (OR) with 95% confidence intervals (CIs) as measures of relative risk of having a blood culture drawn, which was used as a proxy for symptoms of a bloodstream infection. The variable of main interest was calendar year of biopsy. To assess possible linear trends, all variables in the adjusted model were also included, one at a time, as continuous variables and linearity were tested using the Wald test. All models were adjusted for the correlation between biopsies using the sandwich estimator of variance for clustered data (where biopsies from the same man were considered a cluster).

To evaluate whether men undergoing a prostate biopsy experience a higher mortality, we compared the 30- and 90-day mortality rate in the study population with the general population, by calculating age-standardised mortality ratios (SMRs) with 95% CIs.

5 RESULTS

5.1.1.1 Study I

A total of 5 239 men were successfully genotyped. There were 2 542 men with a PSA lower than 10 ng/mL and complete data on the other risk variables. We only calculated the risk of prostate cancer for the first recorded biopsy. In the multivariate analysis all risk factors were associated with the outcome, which was defined as cancer or no cancer at the biopsy (Table 4).

Table 4. Result of multivariate logistic regression of the risk factors for men with a PSA <10 ng/mL (n=2542).

Variable	OR (95% CI)	Cumulative AUC (95% CI)
PSA	1.17 (1.12-1.22)	0.55 (0.53-0.57)
F/T PSA	0.69 (0.66-0.73)	0.60 (0.58-0.62)
Age	1.17 (1.12-1.22)	0.63 (0.60-0.65)
Family History	1.41 (1.29-1.54)	0.64 (0.62-0.66)
Genetic Score	1.52 (1.45-1.59)	0.67 (0.65-0.70)

The logistic regression analyses were used in a prediction model where the outcome gave a risk of being diagnosed with prostate cancer. This was done for the non-genetic, the genetic and a hypothetical genetic model. A distinct cut-off that perfectly separates men with disease from those without cannot be found. Therefore we chose to visualize how many biopsies could be saved at different cut-offs and to what cost in terms of missed aggressive cancers (Table 5).

Table 5. Number of biopsies performed, cancers detected, and biopsies saved at different risk cut-offs for the non-genetic, the genetic, and the optimal genetic model.

Model, %	Performed	No. of saved biopsies (%)	Cancer Detected	Cancer missed No. (%)	Aggressive cancer detected	Aggressive cancers missed No. (%)
Non-genetic	1000	0(0)	365	0(0)	60	0(0)
20	949	51(5.1)	352	13(3.6)	59	1(1.7)
25	871	129(12.9)	338	27(7.4)	56	4(6.7)
Genetic						
20	878	122(12.2)	344	21(5.8)	58	2(3.3)
25	773	227(22.7)	321	44(12.0)	55	5(8.3)
Optimal Genetic						
20	745	255(25.5)	348	17(4.7)	59	1(1.7)
25	686	314(31.4)	340	25(6.8)	57	3(5)

The number of saved biopsies is dependent on the risk one is willing to take to miss cancers. If the threshold for a biopsy lies at 20% the genetic model would save 122 biopsies, at a threshold of 25% 227 biopsies would be saved. The number of aggressive tumours missed when not performing a prostate biopsy on all 1000 men would be 2 and 5, respectively.

5.1.1.2 Study II

A total of 860 men were invited after successfully genotyping 2696 individuals with a PSA between 1 and 3 ng/mL and 192 men were booked for prostate biopsy out of which 172 men underwent the procedure. There were 50, 79 and 43 men in the low-, intermediate- and high-risk group respectively based on their genetic score. In men with low risk, 18% (n=9) were diagnosed with prostate cancer, 28% (n=22) were diagnosed in the intermediate risk group, and 37% (n=16) diagnosed in the high-risk group. We also explored if the SNPs had a discriminatory possibility for intermediate or high-risk prostate cancer, defined as Gleason 7 or higher. In the low-, intermediate- and high-risk group there were 2%, 5% and 12% respectively, but the trend analysis was borderline statistically significant. The result of the multivariate logistic regression is shown in table 6.

Table 6. Results from the multivariate logistic regression analysis. Family history is defined as any first-degree relative with prostate cancer.

Risk factor	**OR**	**95% CI**
Genetic score	1.6	1.05-2.45
Total PSA, ng/mL	1.06	0.72-1.54
f/t PSA	0.98	0.95-1.02
Age, yr	1.08	0.99-1.18
Prostate volume, cm^3	0.95	0.91-0.99
Family history, yes/no	0.73	0.29-1.81

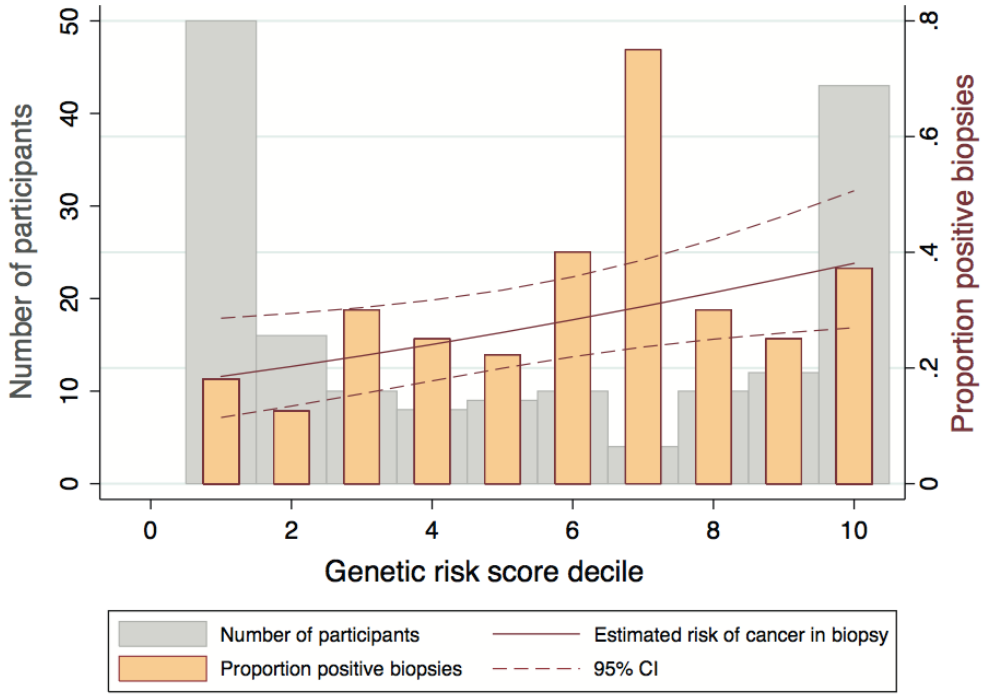

Figure 7.
The estimated risk and observed outcome of positive prostate biopsies in 172 men without a known previous prostate biopsy and a PSA of 1-3 ng/mL, by their decile of genetic risk. (Reprinted with permission, copyright European Urology)

The proportion of positive findings on biopsy correlate with the genetic risk score as seen in Figure 7. The oversampling at the end-deciles is visualised by the grey shaded columns in the figure.

5.1.1.3 Study III

In 2012 there were 133 118 PSA tests performed in a population of 483 807 men older than 40 years of age in Stockholm, Sweden. The number of prostate biopsies was 4694. We noticed a clear increment in the number of prostate biopsies done after a cancer diagnosis, from 233 in 2004 to 582 in 2012, which were interpreted as active surveillance biopsies. Total number of biopsies per year and type was also calculated; the number of primary diagnostic biopsies is presented in table 7.

Table 7. Number of primary diagnostic biopsies performed in Stockholm, Sweden, by year.

	Year of Prostate Biopsy								
	2004	2005	2006	2007	2008	2009	2010	2011	2012
Primary biopsies									
Without cancer	2046	2023	1747	1621	1433	1543	1499	1577	1833
With cancer	1450	1446	1225	1151	1201	1407	1366	1378	1407
Total nr.	3496	3469	2972	2772	2634	2950	2865	2955	3240

Table 8. Estimated proportion of men who underwent a prostate biopsy within one and two years after a PSA test in Stockholm, Sweden, 2004-2012.

		PSA 4-10 ng/mL			
		at 1 year		at 2 years	
Age group, yrs	at risk (no. of men)	Proportion (%)	CI 95%	Proportion	CI 95%
50-59	7853	58	57-59	67	66-67
60-69	20177	45	44-45	55	54-55
70-79	15844	27	26-27	35	34-36

		PSA >10 ng/mL			
		at 1 year		at 2 years	
Age group,yrs	at risk (no. of men)	Proportion (%)	CI 95%	Proportion	CI 95%
50-59	1793	67	65-69	73	71-74
60-69	5770	58	57-59	65	64-65
70-79	6336	38	37-39	45	44-46

To estimate the time from PSA test to a clinical investigation defined as prostate biopsy we performed a survival analysis where an event was defined as either a man being diagnosed with prostate cancer, having undergone a prostate biopsy or had a repeat PSA lower than 4 ng/mL. Men were censored if they migrated away from Stockholm, died, or at the end of study period. We found that for men in ages 50-59 and 60-69 years with a PSA of >10 ng/mL 67%, and 58 %, respectively, had performed a prostate biopsy within one year. The corresponding proportions for men with a PSA of 4-10 ng/mL were 58% and 45%, respectively (Table 8).

As PSA testing is frequent among men living in Stockholm [114], we investigated what proportion of individuals, who were diagnosed with an advanced disease, defined as either T3/T4, N1, M1 or a PSA >20ng/mL, had a pathological PSA registered prior to their cancer diagnosis at certain time intervals for men aged 50-69 years (n=1497). One out of eight of these men had a first recorded PSA above 4 ng/mL more than six months prior to their diagnosis. To visualise the distribution of their first recorded PSA value, we plotted this as a

function of time before diagnosis (Figure 8). Three out of four men diagnosed with an advanced disease did not have a PSA taken prior to their diagnostic PSA test.

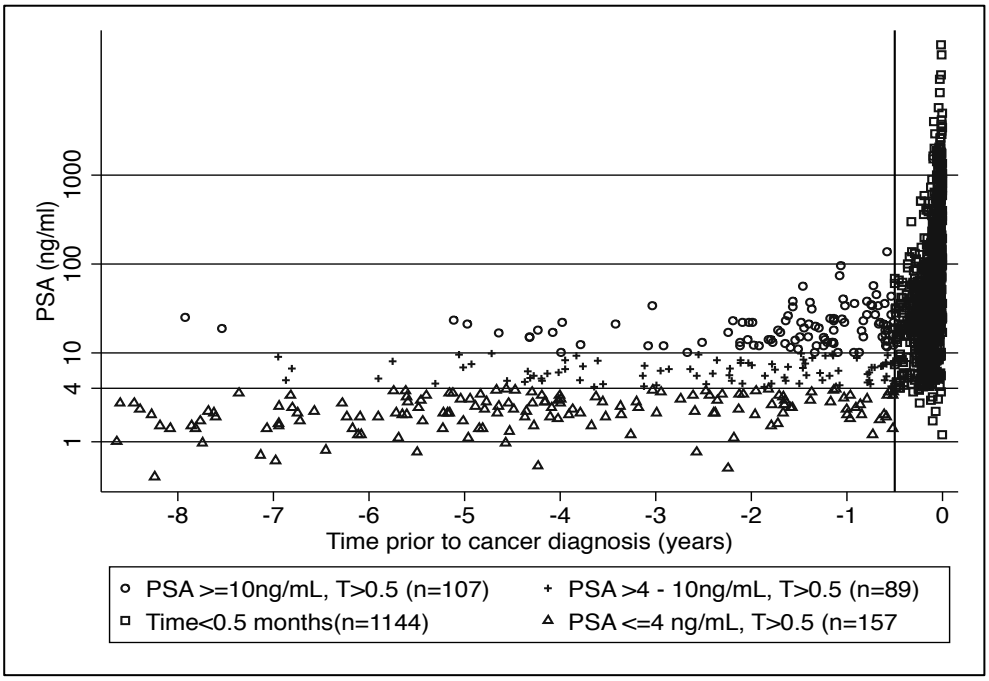

Figure 8.
Plot over first known PSA for men aged 50-69 years at time of diagnosis of an advanced prostate cancer (n=1497).

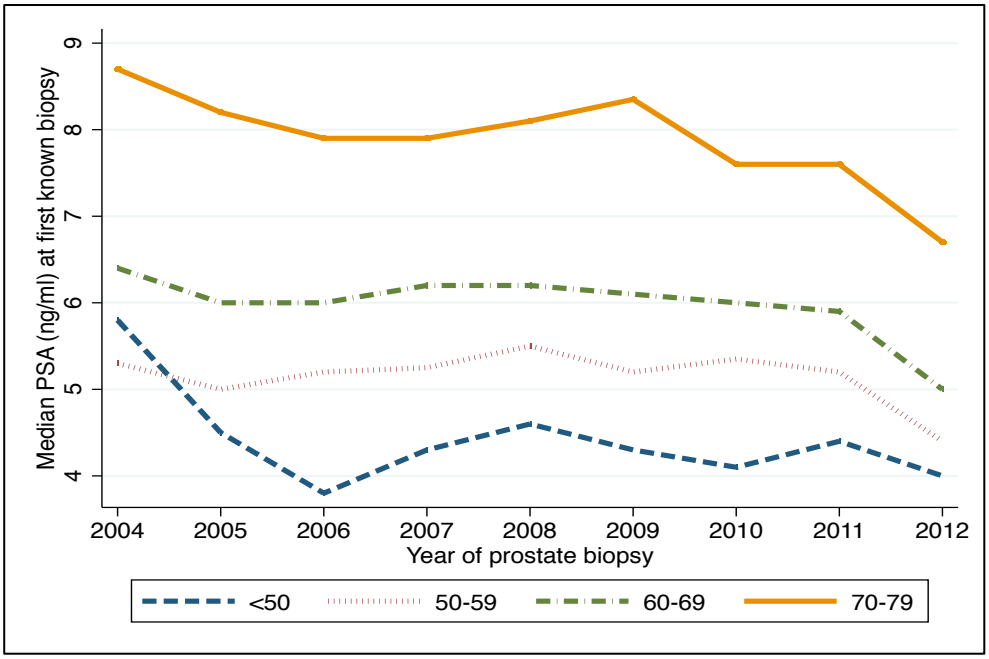

Figure 9.
Plot of median PSA at first known prostate biopsy in Stockholm, 2004-2012, stratified by age category at time of biopsy.

In Figure 9, the median PSA value at the first recorded biopsy is presented stratified by age category at the time of the prostate biopsy and year of biopsy. The median PSA is clearly related to the age of the man undergoing a prostate biopsy. Younger men perform a biopsy at a lower PSA than older men. There is a tendency that the median PSA at the first recorded biopsy is dropping at the end of the study time for all age groups.

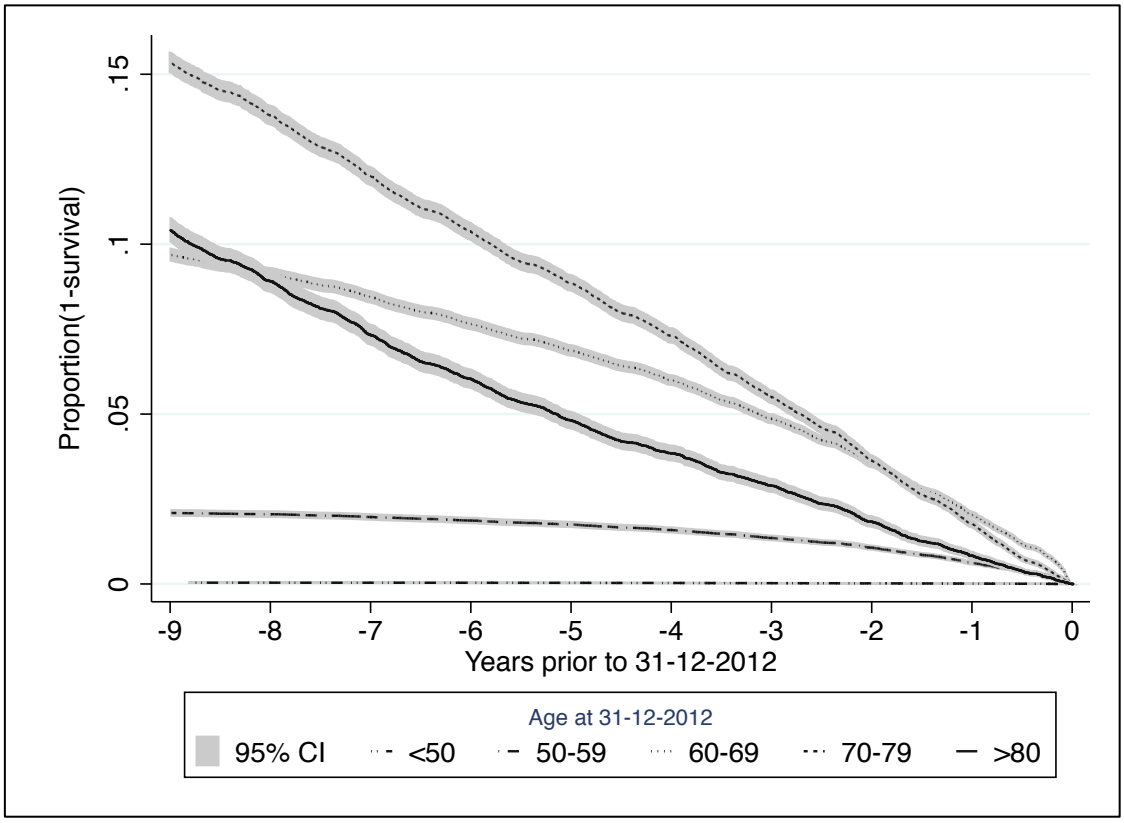

Figure 10.
The cumulative proportion of men who, prior to 31-12-2012, underwent a prostate biopsy, stratified by age at 31-12-2012. This figure includes all types of transrectal ultrasound-guided prostate biopsies (e.g. primary diagnostic, follow-up and active surveillance biopsies).

The proportion of men living in Stockholm who, during the last nine years, underwent at least one prostate biopsy is shown in figure 10. At five years prior to 31-12-2012, 2.3% of men aged 50-59 years had undergone a prostate biopsy, for men aged 60-69, 70-79 and >80 years the proportion is 7.5%, 8.5% and 5%, respectively. As the time prior to 31 December 2012 increases, the proportion of men who has undergone a prostate biopsy increases almost linearly for all ages.

5.1.1.4 Study IV

There were 44 047 prostate biopsies performed in men older than 30 years of age between 2003 and 2012. On 620 occasions men underwent a blood culture within 30 days of the prostate biopsy, out of which 266 were positive. The proportion of men undergoing a blood culture in 2003 was 1.14% whereas the proportion was 2.32% in 2012 and the corresponding proportions for positive cultures were 0.38% in 2003 and 1.14% in 2012. This increase in infectious complications is also reflected by the proportion of men admitted to hospital and discharged with an ICD-10 code related to infection which increased from 1.26% in 2003 to 1.85% in 2012, as seen in figure 11.

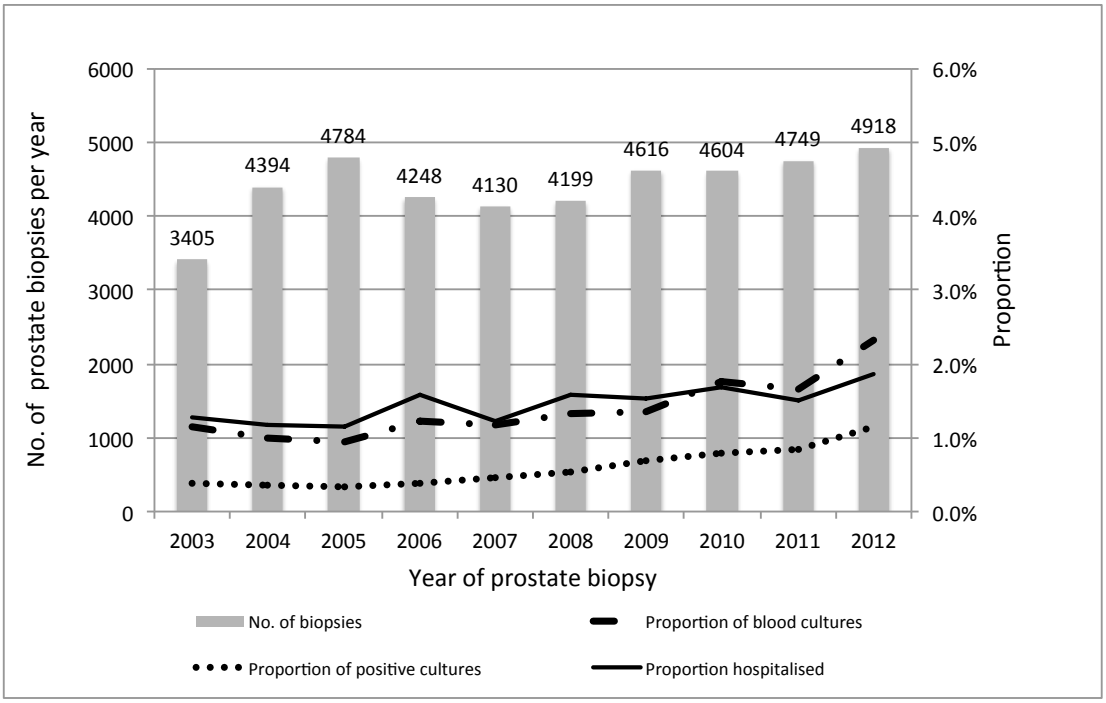

Figure 11.
Number of prostate biopsies and proportion of men undergoing blood cultures, having a positive culture and proportion of men being hospitalised within 30 days of the prostate biopsy performed in Stockholm, Sweden, 2003 to 2012.

The vast majority of blood cultures (75%) were performed within one week of the prostate biopsy (Figure 12). During the study period, the median time for hospitalisation fluctuated around three days although some men were hospitalised for months following a prostate biopsy (Figure 13).

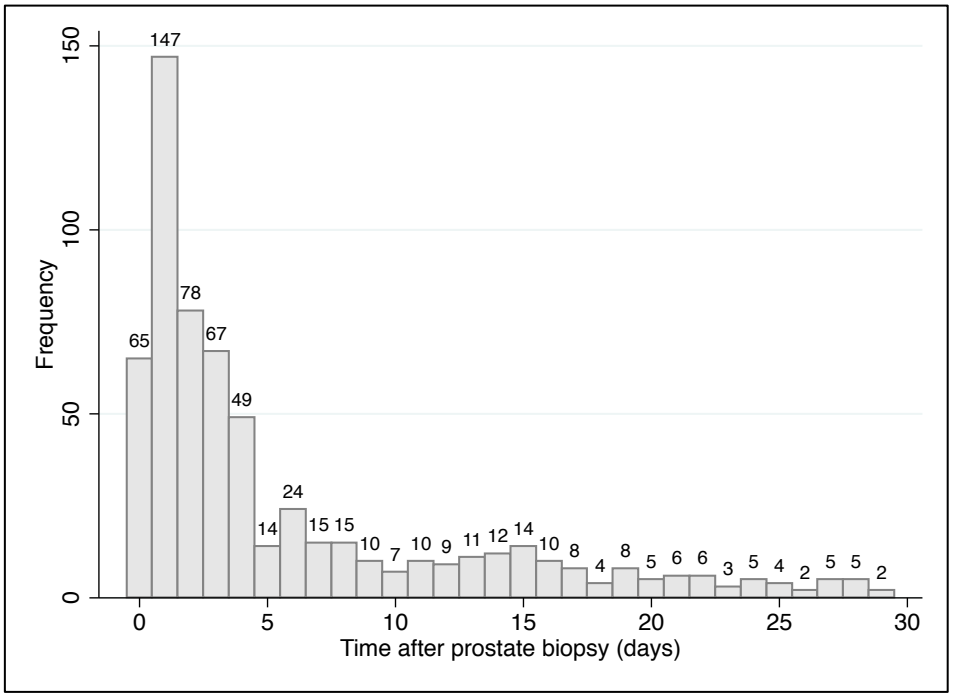

Figure 12.
Time from prostate biopsy to blood culture in days in men who underwent a prostate biopsy in Stockholm, Sweden, 2003 to 2012.

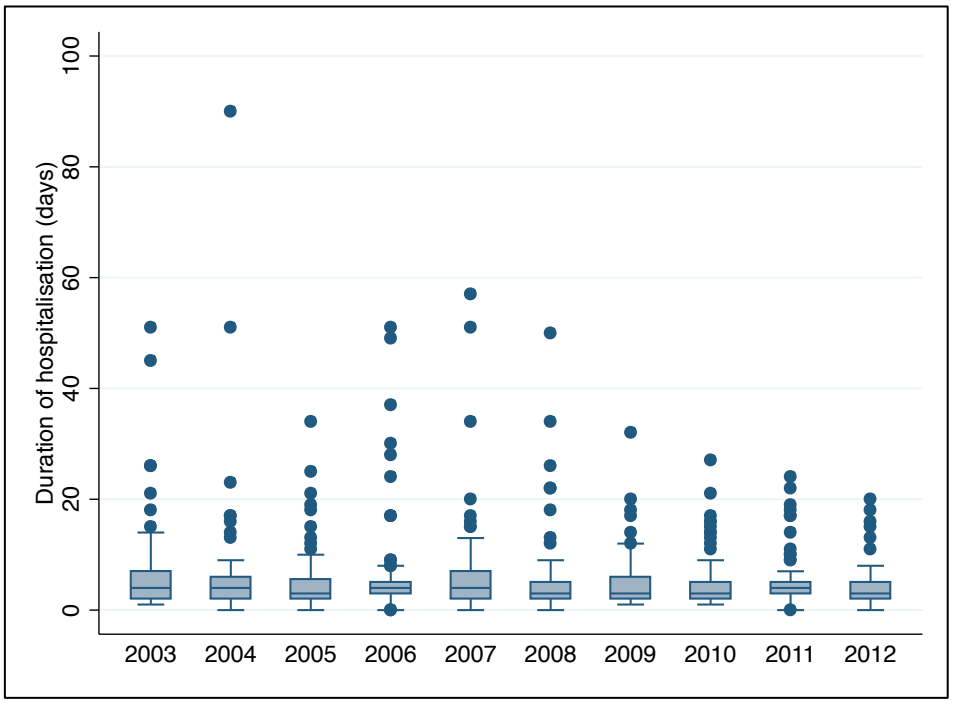

Figure 13.
Boxplot over time of hospitalisation following a prostate biopsy for men hospitalised within 30 days of a prostate biopsy with an ICD code related to an infection, Stockholm, Sweden, 2003 to 2012.

The most common pathogens found in the cultures were E. coli (84.1%), Klebsiella spp (4%) and other enterobacteriacea (4%). There was a clear increase in the proportion of culture with biograms reporting resistant bacteria over the years ($p<0.05$).

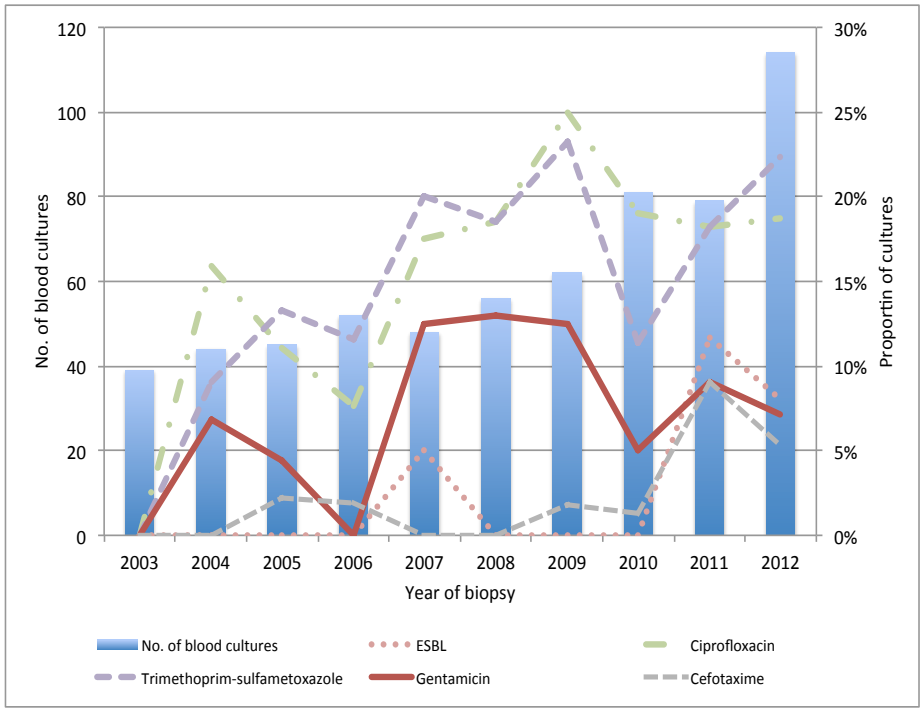

Figure 14.
Number of positive cultures during the study time and the reported proportion of bacteria resistant to specific antibiotics.

In 20% of all blood cultures and in more than 50% of the positive cultures a bacteria resistant to ciprofloxacin or trimethoprim/sulfamethoxazole was found (Figure 14). Ciprofloxacin is commonly used as a prophylactic antibiotic in the prostate biopsy setting.

The level of PSA had no correlation to the risk of undergoing a blood culture or having a positive blood culture. A higher age at the time of biopsy seemed to reduce the risk of undergoing a blood culture after a prostate biopsy. The risk was lower, however, for men who had undergone multiple previous biopsies. The later a biopsy was performed during the study time the higher was the risk of undergoing a blood culture as well as having a positive culture (Table 9). As seen in Table 9, there is no evident difference if the outcome is having a blood culture compared with having a positive blood culture. The CCI is strongly associated with the risk of having a positive blood culture.

Table 9. Adjusted logistic regression analysis reporting OR for men undergoing a blood culture and for those with a positive culture compared with those who did not undergo a blood culture or had a negative culture.

	Having a blood culture		Having a positive blood culture	
Risk factor	**OR**	**95% CI**	**OR**	**95% CI**
PSA, ng/mL	1.00006	0.9998-1.0003	0.9998	0.9989-1.00006
Age, year	0.99	0.98-1.002	0.97	0.96-0.99
CCI	1.78	1.61-1.96	1.67	1.45-1.93
Previous biopsies	0.86	0.75-0.97	0.74	0.60-0.91
Year of biopsy	1.11	1.07-1.15	1.18	1.12-1.24

6 DISCUSSION OF FINDINGS

6.1.1.1 Study I

By adding 35 SNPs to a risk prediction model based on age, PSA, f/t PSA and family history it was estimated that the proportion of biopsies performed can be reduced by between 12.2 to 22.7%.

As with other diagnostic tests, the genetic model is not perfect. The cost of saving biopsies is paid in missed cancers. By setting the cut-off at 20% the genetic model would miss 5.8% of the cancers and, with a cut-off at 25%, 12% would be missed. For non-aggressive cancers this does not necessarily lead to any severe consequences, at least not in the short run, perhaps not even in the long run, since Gleason 3+3 tumours do not seem to have the possibility to metastasise [124].

Under the assumption of a polygenic model and that the sibling relative risk of developing a prostate cancer is 2.5, an optimal genetic prediction model was constructed. This model estimates the predictive value if all genetic information with regards to association of prostate cancer risk in the genome was known. The optimal genetic prediction model would save between 25.5 and 31.4% of biopsies.

However, the number of aggressive prostate cancers missed would increase from 3.3 to 8.3% of the missed cancers at the first biopsy session. Strategies have to be found to take care of those men with a negative first biopsy but with a persistent high risk of aggressive prostate cancer. The Swedish guidelines suggest that these men shall undergo a repeat prostate biopsy within three months [101]. By using MRI or a saturation biopsy model, the detection rate after a first negative biopsy session can be increased [74,133]. It has also been described that genetic prediction model based on SNPs may be of value when deciding if a man should undergo a repeat biopsy or not [134].

Translated to a fictive situation in Stockholm, Sweden, the genetic model would save approximately 250 to 480 biopsies per year at the cost of missing 5 to 11 aggressive cancers at the primary biopsy (based on an average nr. of primary diagnostic biopsies of 3000 per year out of which 2100 are performed in men with PSA <10; combination of Table 7 and Study IV).

6.1.1.2 Study II

Previous studies have shown that men with a low PSA have a significant risk of prostate cancer and also that the proportion of individuals diagnosed with a clinically relevant prostate cancer is not negligible [26,135]. In this study, we showed that a genetic risk score could be used to identify men with low PSA, 1-3 ng/mL, at a higher risk of prostate cancer in. There was a tendency that the men with the highest genetic score were, to a higher degree, diagnosed with a clinically relevant prostate cancer, defined as Gleason 3+4 or higher, that at least would render a discussion of radical treatment. In fact, eleven out of the 47 men underwent radical treatment based on the findings on their first biopsy.

Neither PSA nor f/t PSA could discriminate healthy from ill men in this very selected cohort. Prostate volume seemed to be correlated to the risk of being diagnosed with prostate cancer, where a smaller volume increased the risk.

The value of diagnosing men at an earlier time point is not obvious. There is no doubt that screening does reduce mortality, but some studies have shown that screening for prostate cancer detects the tumour earlier in patients undergoing screening than in the control groups. This lead time varies between 5 and 13 years [136,137]. For men with a disease that demands radical treatment this is most likely positive, but for a large proportion, whose tumours are low grade, this means that they are labelled with a prostate cancer diagnosis and suffer the psychological effects of that. Some of these men will likely undergo treatment with the risk of suffering from the side effects.

For a screening programme to be efficient the test used has to be easily accessible and reasonably priced and the disease has to have a preclinical phase where it is possible to detect it before it has reached an incurable stage. Blood borne markers, such as PSA in combination with SNPs fit these criteria and would most likely work in a screening programme for prostate cancer. An analysis of all SNPs associated with risk of prostate cancer costs approximately 10 euros if done in a routine setting. A PSA analysis costs approximately 8 euros.

Other tests such as DRE and/or ultrasound or MRI of the prostate are, today, either too unspecific or too expensive but might be considered later.

6.1.1.3 Study III

Rates of PSA testing and retesting have been described and the situation in Stockholm, Sweden, is noteworthy: 60% of all men above the age of 60 years have, during the last five

years, had at least one PSA test. This despite the fact that there is no official screening programme. And a large proportion of these individuals were retested within 26 months regardless of their initial PSA [114]. What this testing lead to in regards of transrectal ultrasound-guided prostate biopsies was, however, not clear.

Our study, which counts biopsies performed in men living in Stockholm, shows that an average of 4300 transrectal ultrasound-guided biopsies are done annually in Stockholm, Sweden, in a population of approximately 483 000 men aged 40-79 years.

The transrectal ultrasound-guided biopsy is a frequently performed procedure. During a 5-year period every 13th man aged 60 to 69 years has undergone the procedure and in a 9-year period more than 1/10 in the same age group has done it. When subtracting biopsies resulting in a prostate cancer diagnosis, the proportion is approximately halved – which in the golden setting with a perfect prediction tool could be avoided.

The proportion of men with elevated PSA levels that did not perform a prostate biopsy within one year of the PSA test was unexpectedly high, especially in individuals aged 50-59 years with a PSA >10 ng/mL; one third of these men did not perform a prostate biopsy within one year of their PSA test, which corresponds to 590 men. The causes for this, however, are unknown. The PSA test may have been taken at a time when the patient was suffering from a urinary tract infection and a repeat PSA was analysed which was lower, but not lower than 4 ng/mL as this would have triggered an event in this estimation. Another explanation might be that either a doctor or patient delay was responsible. These results are coherent with an American study where 2/3 of the patients with an elevated PSA had been evaluated after two years of the PSA test. In that study, 18.8% of the men being re-evaluated had a normal PSA, and 32.8% underwent a biopsy [138].

A longer time between PSA and biopsy is correlated with finding more T2c cancers than T2a and T2b in men with a shorter time between PSA and biopsy [138]. In another American study, the time between a PSA test, and response by clinicians to the test, was studied. In 15.8% of the cases, the latency between the test results and the action taken upon them was more than 180 days [139]. In an optimal setting the time between a test and the response to the patient is very short in order to limit the patient's concerns after undergoing a cancer test.

We observed that 196 of the men being diagnosed with an advanced prostate cancer (n=1497) had a first recorded PSA of >4 ng/mL more than six months prior to diagnosis. A total of 107 men had a first PSA >10 ng/mL more than six months prior to diagnosis. How their tumours would have looked if actions had been taken on their first elevated value can only be

speculated about. An Irish study, however, showed that a delay of more than 12 months was significantly correlated to a higher PSA at diagnosis, larger proportion of palpable tumours, and a higher proportion of leading Gleason 4 in the biopsies than for men with a shorter time between PSA and biopsy [140]. Although a lot of men analyse their PSA, approximately 76% of those aged 50 to 69 years have not had a PSA test prior to their diagnosis of advanced disease. At least 16% of men age 60 to 69 and 12% of men age 50 to 59 years would have been examined two years earlier if a standardised follow-up, like the Gothenburg part of the ERSPC, had been used. It is likely that the tumours of these men would have had a different stage at that time.

The mortality rate for prostate cancer in Sweden has declined minimally over the last few years. But it is not comparable to the reduction seen in screening studies. It has been shown that in areas in Sweden where PSA testing is more common the mortality rate is lower compared with areas where the testing is very prevalent [115]. The situation in Stockholm is comparable to the findings in the PLCO trial where only 75% of men with a baseline of PSA >10 ng/mL underwent a prostate biopsy within a year. This has been argued to be one of the reasons that this trial did not show a reduction in mortality since a large proportion of men with high PSAs did not undergo a biopsy and thereby delayed their diagnosis and treatment. It is thus easy to draw a parallel to the Swedish situation and the minute reduction in prostate cancer mortality although the frequency of PSA testing is high.

The conclusions from this study is that a transrectal prostate biopsy is a common procedure, and that there is room for improvement in several areas. First, the time from a PSA test to a performed biopsy is long, second, a large proportion of men with elevated values do not undergo the diagnostic procedure at all.. One solution to these problems may be structured testing and follow-up.

A drawback of our study is the retrospective design and the lack of knowledge concerning the true reasons why men with an elevated PSA did not undergo a prostate biopsy.

6.1.1.4 Study IV

During the ten years of the study, 44 047 prostate biopsies were performed and 620 were followed by a blood culture within 30 days of the biopsy. The majority of men underwent the blood culture within seven days; 266 of the cultures were positive.

There is a clear effect of time as expressed by the OR for year of prostate biopsy. This effect was prominent, and in 2012 more than 1 out of 50 men who performed a prostate biopsy

received symptoms suggestive of bloodstream infection compared with 1 out of 100 in 2003. These results are in line with the findings of Lundström et al. who described an increased rate of urinary tract infections and hospitalisations after a prostate biopsy over the years [84]. Their study, however, only covered men with a known prostate cancer and they used prescribed antibiotics as a proxy for urinary tract infection. In our univariate analysis, men with a prostate cancer seemed to have an increased risk (OR 1.22) of undergoing a blood culture within 30 days compared with men with no known prostate cancer, an effect which was not seen in the adjusted model most likely due the effect of the comorbidities.

Not only did the proportion of men undergoing a blood culture increase but also the proportion with a positive blood culture, which tripled during the study period. This is most likely attributed to a change in virulence of the bacteria interpreted as a loss of sensitivity to the prophylactic antibiotic given at the time of the prostate biopsy.

From the resistance patterns of the positive blood cultures we could see that the proportion of cultures with ciprofloxacin-resistant bacteria has increased substantially over the last few years. The same is seen for trimethoprim/sulfamethoxazole. Both of these antibiotics are commonly used as a prophylaxis before ultrasound-guided biopsies of the prostate.

The rate of hospitalisations almost doubled during the time frame studied. This is interesting, as the number of beds at hospitals in Sweden for surgical care has dropped from 18 000 in 1986 to 7000 in 2013. Sweden has one of the lowest numbers of hospital beds per capita in Europe. This means that if the numbers of infections continue to increase this will have a push-out effect on patients treated for other conditions or planned for other procedures [141,142]. The median time for hospitalisation, ≈3 days, after a biopsy-related infection did not change significantly during the study time.

From an international perspective Sweden has been relatively spared from antibiotic resistance in microbes [95]. This is perhaps due to the historically low use of antibiotics in Sweden [143]. The belief that a frequent use of antibiotics leads to an increase of multi-resistant bacteria has led to an information campaign for Swedish doctors and the prescription rate for outpatient use of fluoroquinolones has decreased by 17-27% over the last 6 years [144].

The reason for the increase in resistant strains in Sweden is likely multifactorial. One reason is probably increasing travel to foreign countries. Up to 24% of healthy volunteers that travelled outside Northern Europe were colonized with ESBL-producing Enterobacteriaceae upon arrival in Sweden [145]. It is unclear for how long these individuals carry the bacteria.

As a comparison it has been shown that up to 46% of patients infected with an ESBL-producing Enterobacteriaceae carry the resistant strain for up to one year after infection [146]. This indicates that recent travel has to be considered when choosing the proper prophylactic antibiotics, especially if the patient is suffering the clinical symptoms of an infection.

The results in this study regarding the situation in Stockholm probably reflect a best-case scenario where the prescription rate of antibiotics in the community is low and decreasing and the prophylactic regimens before biopsies are reasonably stringent and sparse. From an international perspective these rates are fairly low, but for an urologist practising in Sweden the development of multi-resistant bacteria is a fearsome prospect.

7 METHODOLOGICAL DISCUSSIONS

In classical epidemiological research, where causes and diseases in populations are studied, several different types of studies can be conducted. In this thesis we conducted cohort studies.

A cohort study is a longitudinal study, which follows study subjects free from the disease at the start of the study and observe who develops the disease within the study period. They are thus defined by their exposure and followed up for the outcome. The common denominator for those included may be year of birth, place of birth, city they live in and so on. The study subjects may also be chosen by a certain common exposure, such as PSA testing or transrectal ultrasound guided prostate biopsies in our studies.

Cohort studies have the advantage of making it possible to calculate incidence rates and absolute risks for a certain outcome. A cohort study may also investigate more than one outcome.

No study is without limitations or errors. The following sections will cover some of these aspects and put the studies in this thesis into this context [147].

7.1.1.1 Non -systematic vs. systematic errors

Non-systematic errors

Non-systematic errors refer to random errors, which occur in all types of studies. They may occur during all stages of a study and include errors such as transforming an answer in a questionnaire to a dataset and an answer is wrongly typed in as “1” where it should have been “0”. Usually the errors are of minor importance and in most studies they also occur randomly

occurring in both the cases and controls. These types of errors cannot be corrected for in the statistical analysis. A large sample size usually reduces the influence of random errors.

Systematic errors

Systematic errors refer to a situation where the error affects the analysis in a non-random way. There are mainly three types of systematic errors of interest, information bias, selection bias and confounding. Subgroups to these exist and are exemplified below with relevance to the studies in this thesis.

7.1.1.1.1 Information bias

Misclassification

Misclassification refers to the situation when the collected information is incorrect. This type of information bias can be subdivided into differential and non-differential misclassification. The former describes the situation where information on a certain exposure is classified differently among those with the disease and those without the disease. The association found could either be directed towards the null or away from the null. In non-differential misclassification, this error is not associated with the outcome and is thus usually of less importance since it mainly dilutes the estimates toward a null result, possibly leading to a false negative finding. By using large validated registers these types of error were reduced.

Recall bias

When studying subjects retrospectively and collecting information regarding exposure to risk factors the subjects who developed the disease of interest may remember exposures differently than those who did not developed it. As an example of this in our studies we collected exposure status with regards to family history of prostate cancer. Those men who developed prostate cancer may have had more time and interest to discuss these topics with family members and recollect information regarding the disease status and, thereby, report that in a higher degree than those who were not diagnosed.

This may be the case for Studies I and II where information regarding family history was used in the prediction model. It is very difficult to estimate how large this effect is in this type of study.

Selection bias

Selection bias in retrospective cohort studies may be introduced when subjects in one of the exposure groups are more or less likely to be selected if they had the outcome that is studied. One form of selection bias might be present in Study IV where the mortality rate in men undergoing a prostate biopsy is lower than that for the general population for men younger than 70 years of age. The selection of men undergoing a prostate biopsy is made by the doctors performing the procedure, if a man with severe comorbidities show up for a doctors appointment to discuss whether or not to investigate an elevated PSA the doctor might be inclined to recommend the patient not to undergo the procedure as other illnesses could be of larger concern – thereby selecting only men healthy enough to undergo the biopsy and since they are healthier than the average their mortality rate following a prostate biopsy is lower. This means that the control population for such calculations has to be chosen carefully not to miss any substantial negative effects of the procedure. In the case of study IV the control population chosen was the age-matched reported mortality rates for all males in Sweden. We considered choosing to calculate the mortality rate for men undergoing a PSA test in Stockholm to compare with our cohort, but we argued that PSA testing is so common in Stockholm that this would not have made any difference.

Men lost to follow up could also raise concerns regarding a cohort study. Bias, as an effect of this, may occur if there is a difference in loss to follow up in those exposed and those not exposed. If men at random, regardless of their exposure status, are lost to follow up this does not introduce bias.

7.1.1.1.2 Confounding

A confounding effect is when there is a second exposure that is both associated with the primary exposure of interest and the outcome, but the secondary exposure is not a direct link between the primary exposure and the outcome. Confounding may cause both an over- and underestimation of the effect of the primary exposure. There are ways of controlling for confounding. The best way in a prospective cohort study is to randomize study subjects to the different study arms. This leads to the confounding factors, both known and unknown, being likely to be evenly distributed amongst the different study arms. This is however not possible to do in the retrospective setting. In these studies, the confounding effect may be reduced or controlled by stratification of the study subjects, for example by age and/or sex. The risk for the outcome is then analysed in different strata. However there is no guarantee that other confounders than the one stratified for are controlled for. The residual confounding could be due to incomplete information regarding the confounding variables or unknown confounders

that were not controlled for. Another reason might be that the stratification is to coarse or even wrong.

Another way to control for this is to do regression analysis where simpler, unadjusted, models are compared to more complex, adjusted, models. How confounders or different exposures interact can be estimated by introducing the different variables step by step into the regression analysis and seeing how the results differ. It is important to recognise that even after good adjustments of the model there will be residual confounding.

The logistic regression analysis done in Studies I, II, and IV use these methods. Age is an example of confounding in study IV, where a higher age is associated with the risk of having a positive blood culture in the unadjusted model, but when adjusting the model with Charlson Comorbidity Index the age effect vanishes for older men. This was interpreted as if older men have a risk of having more severe diseases, which predisposes to a severe infection. and, thereby, it is not the age itself which is a risk factor but the comorbidities the older man has that is the true risk factor for attaining a severe complication to a prostate biopsy.

7.1.1.2 Validity

Validity refers to the question if the study measures what it is intended to study or not. It is usually split into two different parts, internal and external validity. The internal validity covers the question if the researcher can answer the research question with the presented factors available and if the right study design has been used. A study with a high internal validity has few or no systematic errors or they have been controlled for. External validity or generalizability refers to how well the results of the trial can be used in other populations or settings. The external validity is dependent on the internal validity. In Studies I, III and IV we used data on historical PSA values where there were missing values between 2003 to 2006 from a specific region in Stockholm. These tests represented 15% of the tests performed in Stockholm during these years. Sensitivity analysis was performed to objectify what impact these missing values had on the analysis. By restricting the analysis to other years this effect could be estimated. The restriction did not alter the results significantly. This suggests that the internal validity with regards to PSA is high and the results are likely to be generalisable.

7.1.1.3 Statistical hypothesis testing

Associations between risk factors and outcome may be a result of chance. To demonstrate that a finding is not merely a result of chance a statistical hypothesis testing is performed. To do this a null-hypothesis is compared with an alternative hypothesis. The null hypothesis is usually formulated as if there is no difference between two compared groups whereas the

alternative hypothesis claims that there are differences. The next step is to set a limit, p (a probability threshold), if the test is lower than this the null-hypothesis may be rejected. This limit is usually set at 1 or 5%. The statistical tests are then performed to decide whether or not the null-hypothesis can be rejected or not. If the null-hypothesis can be rejected the alternative hypothesis is then possible to accept –but it does not automatically state that the null-hypothesis is wrong – just that it is not probable under the pre-set requisites.

Two types of errors can be encountered in hypothesis testing. If the null-hypothesis is true and rejected a type 1 error (false positive) is committed. A type 2 error is committed if the alternative hypothesis is true and rejected (false negative).

7.1.1.4 Power calculations

In order to estimate how large a study has to be in order to detect statistical significant differences between studied groups a power calculation is performed. Statistical power is inversely related to the probability of making a type 2 error. If the statistical power is high the risk of doing a type 2 error is small. As the study is not done yet some assumptions have to be made. Firstly, an estimation of how large the difference, or effect size, may be is done. The larger the difference the smaller study is needed. The information regarding the effect size is often not known before the study but results from similar studies can be used to estimate this. The next decision that has to be made is how large the chance should be that one is willing to accept for the study to result in a conclusion that is incorrect. This limit is almost always chosen at 0.05, which is the probability threshold of making a type 1 error, also called the α-criterion. The next step is to estimate how large the probability should be to detect a true difference; this probability is commonly set to 0.80. After these steps are taken a power calculator can be used to estimate the sample size needed. Depending on the study planned different formulas is used to estimate either the power or the study size needed.

When study subjects are invited to participate there will always be some who do not want to or cannot participate which means that a slightly larger number of people than are really necessary according to the power calculation need to be invited.

In this thesis we used power calculations to estimate how many men needed to be invited in Study II in order to find a statistical significant difference in the groups. Since prostate biopsies carry a certain risk for complications it was of great importance to reduce the number of men undergoing the procedure. By oversampling at the end-deciles, that is men with the lowest and highest genetic risks, we were able to reduce the number of men invited and, thereby, limit the number of prostate biopsies performed.

8 CONCLUSIONS

- Genetic markers can be used to avoid unnecessary prostate biopsies in men with moderately elevated PSA levels
- Genetic markers can be used to identify men at higher risk of prostate cancer although their PSA level is low
- More than one third of the men with a PSA >10 ng/mL do not undergo a prostate biopsy within one year of the PSA test
- The proportion of men suffering from severe infectious complications after prostate biopsies has increased during the last 10 years

The diagnostic pathway for prostate cancer in Stockholm is suboptimal. A large proportion of men with a high suspicion of prostate cancer do not undergo a diagnostic procedure within a reasonable period of time.

By using an SNP-based genetic risk score as a complement to the common markers used today men at higher risk who do not undergo prostate biopsies today, since their PSA is low, would have a chance of being treated earlier. By complementing the PSA test with genetic markers, a substantial proportion of biopsies could be spared The use of genetic markers as a complement to PSA would also reduce the number of men suffering from severe side effects of the prostate biopsy since they would not have to undergo the diagnostic procedure.

Implementing a structured protocol for prostate cancer testing based on genetic markers and PSA would likely improve the current situation.

9 FUTURE PERSPECTIVES

Prostate cancer is of great relevance to both society and to the people affected by the disease. The disease is not only the most common solid malignancy among men in Sweden today but also one of the most costly to treat. There are three areas regarding prostate cancer where efforts are made and have to be intensified: pre-diagnostic, diagnostic and treatment.

The pre-diagnostic area includes screening for prostate cancer. When will screening be introduced in Sweden? The question is not if but rather when. There is evidence to suggest that screening does lower the mortality rate of prostate cancer, but how a screening programme should be implemented is not clear. In the ERSPC, different countries used different screening intervals but only used PSA as a trigger for biopsy. With the introduction of new markers it would be possible to individualise the screening interval in order to optimise testing. Men with a low risk of prostate cancer may be screened with longer intervals. Men with a higher risk, but not reaching the cut-off for a diagnostic test, can be screened with shorter intervals. This screening protocol would be complex but would be of benefit not only for the people paying for the screening but also for the men undergoing it. Not only will new markers be used in the screening situation but also in the setting of repeat biopsies in men with an increased risk of prostate cancer. New diagnostic and prognostic markers will enhance the selection of men who need to undergo repeat biopsies. During the fall of 2013 the STHLM-3 study was initiated, in which the primary goal is to reduce the number of unnecessary prostate biopsies with retained sensitivity for Gleason 7 tumours. By combining the predictive information in SNPs and a number of biochemical markers, such as PSA and hK2, the hypothesis is that this can be achieved. In so far more than 60 000 men have been invited and 4 000 have undergone a prostate biopsy. The last letter of invitation was sent in November 2014 and the preliminary results of the study will likely be presented during 2015.

Regarding the diagnostic area, the prognostic information based on the histological architecture of the tumour is fairly good but the process is slow and to some extent inaccurate. There is also a problem with interobserver variability. Then there is an uncertainty as to whether or not the most aggressive tumour is sampled or not when analysing prostate biopsies. To address the problem of correct sampling, better approaches to identify the most aggressive tumour in the prostate have to be evaluated. In an effort to do this our research group is planning a MRI fusion-guided biopsy study during 2015. A paired design study

approach will be used where men with a high risk of prostate cancer are identified through the STHLM-3 biomarker panel and invited to undergo a MRI of the prostate. All men will undergo a regular 12 core prostate biopsy and those men with a suspect lesion at the MRI will undergo directed biopsy as well. Hopefully this will show that MRI can be a part of the diagnostic procedure in a screening programme. There is some data suggesting that MRI improves the detection of high-grade prostate cancer, meaning that men with no evident tumour on MRI do not need to undergo prostate biopsies.

To address the interobserver variability and the relatively slow process of the histological examination, the great advances in genomic research will likely be useful. In a short period of time it will be possible to do genomic profiling of prostate tumours as a part of the histological evaluation of the specimens, and not only to look for specific mutation but the whole tumour genome. The information on the individual mutations will most likely give a good answer to the prognosis of the individual prostate cancer in an individual. There is some evidence that genomic profiling can be translated to a Gleason Score and thereby contain the same prognostic information. If this holds true it means that the diagnosis and prognosis for the prostate cancer can be obtained within a couple of days of the biopsy at a reasonable cost. By using standardised laboratory analysis equipment the problem of interobserver variability could be minimised. This technique is being evaluated for breast and colon cancer and within a year the process for prostate cancer will be ready.

One of the largest problems within the field of medicine for mankind lies ahead of us. The increasing problem with multi-resistant bacteria will cause severe harm to people in the future. In the worst case scenario the future will be like the past when there were no antibiotics. On a global scale the use of antibiotics must be decreased. This can only be done through information and education. Although prostate biopsy is a common procedure this is not the main driver behind the increasing antibiotic resistance. The urological community can contribute by reducing the number of men who need to perform prostate biopsies. An individualised prophylactic regime will most likely be used. Before the biopsy a stool sample will be sent in and analysed with regards to pathogens in the rectum and their resistance pattern. By using this information each patient will receive an individualised antibiotic prophylaxis at the time of the prostate biopsy. Another approach is to reduce the number of cores taken and to disinfect the needle between each core. During 2015 a large study will be undertaken in our group where the biopsy needle will be rinsed in formaldehyde between each core. A small study has been done in Canada showing that infection rate could be reduced to one third of its current level with this simple routine.

Over a longer perspective, liquid biopsies will be performed in blood or urine or a combination of these. By analysing circulating fragments of DNA or tumour cells in the blood or urine a diagnosis can be set. This can also be used to identify men who after surgical treatment might be candidates for adjuvant radiation therapy due to positive surgical margins.

Prostate cancer treatment has been conservative. In breast, bladder and colon cancer treatment the patients are offered multimodal treatments with better results. This must be the case for prostate cancer as well. Randomised trials with either docetaxel or abiraterone in combination with surgery will be of great interest, with our without the adjuvant radiotherapy for advanced cases. Perhaps genetic profiling of the tumour will be of benefit here to identify those tumours that are sensitive to a specific treatment.

10 POPULÄRVETENSKAPLIG SAMMANFATTNING

Prostatacancer är den vanligaste tumörformen bland män i Sverige. Varje år diagnostiseras 1800 män i Stockholm och ca 1100 genomgår botande behandling. Antalet män med nyupptäckt prostatacancer låg från mitten av 1950-talet till början av 1990-talet relativt stabilt, liksom antalet som dog till följd av sjukdomen. I mitten av 1990-talet kom cancermarkören PSA som snabbt etablerades som ett test för att bedöma risken för huruvida en man hade prostatacancer eller inte. Detta ledde till att antalet män som diagnostiserades med prostatacancer ökade kraftigt, däremot låg dödligheten kvar på i princip samma nivå. Under senare år har dödligheten i prostatacancer minskat minimalt.

Det finns tre etablerade riskfaktorer för prostatacancer. Dessa är ålder, familjehistoria och etnicitet.

Det har diskuterats om screening för prostatacancer ska införas i Sverige. Två stora internationella studier, ERSPC och PLCO, har visat olika resultat avseende screening. I ERSPC minskade dödligheten i prostatacancer med 20 %, medan den amerikanska undersökningen inte kunde påvisa några fördelar med screening. En av baksidorna med screening är att många av de män som diagnostiseras med prostatacancer har en tumör som i de allra flesta fall inte leder till att mannens liv förkortas, även i frånvaro av behandling. För att undvika att en man dör i prostatacancer måste 293 män bjudas in till screening. För att rädda en man från att dö i prostatacancer måste 8-10 män genomgå behandling.

Prostatacancer diagnostiseras oftast till följd av att en man har låtit undersöka sitt PSA-värde. Är detta måttligt förhöjt, 3-10 ng/mL, har en man en risk på ca 25 % att diagnostiseras med prostatacancer. Är värdet kraftigt förhöjt, mer än 50 ng/mL har nästan alla män en prostatacancer. Det finns dock inte något strikt normalvärde för PSA; även män med värden mellan 1 och 3 ng/mL, som idag anses ligga inom normalvärdet, har risk för prostatacancer.

PSA testning är vanligt i Stockholm, mer än hälften av alla män äldre än 60 år har gjort provet under den senaste femårsperioden. Sex av tio män har upprepat provtagningen inom två år oberoende av det ursprungliga värdet. Däremot är det relativt okänt hur många av dessa provtagningar som leder till att män genomgår vävnadsprovtagning och också hur lång tid en man får vänta från det att PSA värdet är taget till dess att en vävnadsprovtagning är genomförd.

Under senare år har en rad nya markörer för prostatacancer utvecklats. Bland dessa märks Hk2 (Humant Kallikrein 2), vilket är en proteinmarkör, samt genetiska basparsvariationer, s.k. SNPs (Single Nucleotide Polymorphisms). De senare är genetiska markörer vilka kan mätas i blodet. Dock har ingen av dem har ännu nått klinisk användning i Sverige.

Män med förhöjd risk för prostatacancer rekommenderas ofta vidare utredning. Hos de flesta män innebär detta att en urolog bedömer prostata genom att känna på den samt att vävnadsprover av prostata tas via ändtarmen. Dessa prover innebär vissa risker för infektion och blödning. Under senare år har risken att behöva sjukhusvård på grund av infektioner ökat efter vävnadsprovtagning av prostata.

Avhandlingens syfte var att undersöka om genetiska markörer kan användas som ett komplement till PSA för att öka träffsäkerheten vid utredning av män med ökad risk för prostatacancer. Målet var också att undersöka huruvida dessa genetiska markörer kan användas för att identifiera män med låga PSA-värden som idag oftast inte rekommenderas vidare utredning, men som trots allt har en ökad risk för prostatacancer,.

Utöver detta beskriver avhandlingen hur stor andel av män i Stockholm som har genomgått vävnadsprovtagning och hur lång tid efter PSA provtagningen en sådan undersökning görs. Därtill undersöktes hur stor del av männen som drabbades av allvarliga infektioner till följd av vävnadsprovtagningen och huruvida denna typ av komplikationer har ökat under det senaste decenniet.

10.1.1.1 Studie I

Till följd av att det i en svensk studie visade sig att man kunde identifiera män med kraftigt förhöjd risk för prostatacancer baserat på deras familjehistoria, PSA och fem av männens egna basparsvariationer ville vi undersöka om dessa genetiska basparsvariationer kunde användas för att bättre identifiera vilka män som behöver genomgå utredning för misstänkt prostatacancer. I studien bjöds män yngre än 80 år in som hade genomgått en vävnadsprovtagning av prostata mellan år 2005 och 2007 vid två av tre laboratorier i Stockholm. Dessa män fyllde i en kort enkät och lämnade blodprover där deras genetiska basparsvariationer analyserades. För att samla information om männens tumörer hämtades information från det svenska cancerregistret och det nationella kvalitetsregistret för prostatacancer. Via de laboratorier som gör PSA-analyser kunde resultatet från dessa hämtas för varje enskild man och provtagningstillfälle, vilket också bildade basen i STHLM-0 (ett register över alla män i Stockholm som vid minst ett tillfälle sedan 2003 har gjort ett PSA test).

När den genetiska information lades till den ursprungliga modellen bestående av PSA, en variant på PSA(f/t PSA), ålder och familjehistoria förbättrades förutsägelsen om vilka av männen som hade prostatacancer. I en klinisk vardag innebär detta att doktorer bättre kan förutse vilka som behöver genomgå en vävnadsprovtagning av prostata. I studien visades att upp till 23 % av vävnadsprovtagningarna kunde undvikas utan att för många fall av allvarlig prostatacancer missades.

10.1.1.2 Studie II

Från en stor amerikansk studie är det känt att män med PSA strax under 3 ng/mL har lika stor risk för prostatacancer som män med PSA mellan 3 och 10 ng/mL. Ur detta perspektiv var syftet med studien att belysa huruvida genetiska basparsvariationer kan användas för att identifiera män med hög risk för prostatacancer trots låga PSA värden. Från en stor studie med 26 000 män i Stockholm som lämnat blod och fyllt i en enkät identifierades män med ett PSA mellan 1 och 3 ng/mL som inte hade någon känd prostatacancer, inte hade genomgått någon tidigare vävnadsprovtagning och var mellan 50 och 69 år. Sammanlagt bjöds 860 män, från tre olika riskgrupper baserat på deras genetiska riskpoäng, in till studien och 172 av dessa genomgick vävnadsprovtagning. Hos de med lägst risk hittades prostatacancer hos 18 %, hos de med mellanrisk hittades cancer hos 28% och hos de med högst genetisk risk hittades prostatacancer hos 37%. Resultaten tolkades som att den genetiska profilen kan användas för att identifiera de män som har högst risk för prostatacancer trots att deras PSA värde är lågt.

10.1.1.3 Studie III

Utifrån kunskapen om att PSA testning är mycket vanligt i Stockholm undersöktes hur vanligt det är att män med förhöjda PSA värden genomgår vävnadsprovtagning av prostata. I en annan svensk studie är det visat att områden i Sverige där PSA testning är vanligt har lägre dödlighet i prostatacancer än områden där testning inte är lika vanligt. Dock är dödligheten i prostatacancer i de områden som har mest PSA testning fortfarande betydligt högre än i den europeiska screeningstudien för prostatacancer.

Målet med studie 3 var att beskriva hur stor andel av männen i Stockholm som har genomgått vävnadsprovtagning av prostata och hur lång tid det tar från ett PSA test till ett vävnadsprov.

Via de laboratorier som analyserar vävnadsprover från prostata identifierades män som mellan 2003 och 2012 genomgått dessa undersökningar. Via STHLM-0 kunde de PSA prov som låg till grund för vävnadsprovet identifieras och även de prov som låg före och efter i tid. Genom att länka männen till det nationella patientregistret kunde vävnadsprovtagningar från

andra typer av undersökningar och eller operationer sållas bort. För att kunna uttala sig om situationen i Stockholm och om hur män boendes i Stockholm utreds länkades männen också till befolkningsregistret varvid biopsier gjorda i Stockholm på stockholmare kunde sorteras fram. Vidare länkades dessa män till det svenska cancerregistret och det nationella kvalitetsregistret för prostatacancer.

Sammanlagt görs nästan 4500 vävnadsprovtagningar av prostata via ändtarmen varje år i Stockholm. Nästan 15 % av alla män mellan 70 och 79 år har genomgått undersökningen. Nästan 10 % av männen mellan 60 och 69 samt 7 % av männen mellan 50 och 59 har genomgått undersökningen under 9 år före 2012.

För män mellan 50 och 59 år med ett PSA värde mellan 4 och 10 ng/mL hade 42 % inte genomgått ett vävnadsprov inom ett år från det att de tog sitt PSA värde. För män mellan 60 och 69 år låg andelen på 55 %. För män med ett PSA över 10 ng/mL hade 33 % av männen mellan 50-59 år och 42 % av männen mellan 60 och 69 år inte genomgått undersökningen inom ett år. Detta var förvånansvärt höga siffror. Vad som händer med männen som inte genomgår utredningen är oklart, men en farhåga är att de inom några år kommer med symtom på obotbar prostatacancer. Detta exemplifierades även med en analys av just de män som diagnostiserades med en avancerad prostatacancer; det visade sig att nästan 13 % av dessa hade minst ett förhöjt PSA värde taget mer än 6 månader före sin diagnos.

Studien belyser att en stor del av män i Stockholm har genomgått denna typ av undersökning. Ungefär hälften av männen har genomgått undersökningen utan att diagnostiseras med cancer vilket indikerar att en stor del av männen genomgår utredningen i onödan vilket i sin tur är ett bevis på PSAs dåliga träffsäkerhet. Utöver detta visade det sig också att det för mer än en tredjedel av män i åldrarna 50-59 år med kraftigt förhöjda PSA värden tar det längre tid än ett år innan de genomför en vävnadsprovtagning av prostata, om de överhuvudtaget genomför undersökningen.

Uppenbarligen är viljan att ta PSA prov stor, men uppföljningen av förhöjda värden ter sig suboptimal. Att införa strukturerad uppföljning skulle kunna förbättra denna situation.

10.1.1.4 Studie IV

Det vanligaste sättet att diagnostisera prostatacancer är genom att ta vävnadsprover av prostata via ändtarmen med hjälp av ultraljud. Det är ett välkänt faktum att detta medför risker för blödning och infektioner. I en nyligen publicerad svensk artikel visades det att ca 6 % av män med prostatacancer som genomgått en vävnadsprovtagning hämtar ut ett recept på urinvägsantibiotika inom 30 dagar efter provet. Andelen män som läggs in på sjukhus har

också ökat sedan år 2006. Flera internationella studier har påvisat en ökad frekvens i befolkningen av multiresistenta bakterier samt att andelen män som genomgår prostatapunktion drabbas av infektionsrelaterade komplikationer ökar över tid. Efter att i den kliniska vardagen ha noterat att ökningen av allvarliga infektioner också skedde i Stockholm gjordes denna studie för att bekräfta dessa farhågor och för att försöka få en förståelse till varför denna ökning har skett under de senaste tio åren. Den allvarligaste infektionskomplikationen till vävnadsprovtagning av prostata är blodförgiftning. Ett tecken på att en man drabbas av detta är hög feber och frossbrytningar. Vid dessa symtom brukar läkare genomföra en blododling för att kontrollera om bakterier finns i blodet eller inte.

Genom att via register över genomgångna vävnadsprover och länkningar till ett flertal svenska hälsovårdsregister kunde 44047 genomgångna vävnadsprover identifieras mellan åren 2003 och 2012 hos 32196 män. Vid 620 tillfällen genomgick män en blododling inom 30 dagar efter provtagningen. Vid 266 tillfällen växte det bakterier i blodet. I början av studieperioden genomgick 1 av 100 män en blododling inom 30 dagar, medan 1 av 50 män gjorde det i slutet av studieperioden. Andelen blododlingar där det växte bakterier ökade från 0,38 % till 1,14 % under samma tidsperiod. Det visade sig att bakterier med nedsatt känslighet för vanliga antibiotika, som ges som förebyggande medicin vid vävnadsprovtagningen, ökade från 2003 till 2012. En teori till ökningen är att dessa resistensmekanismer är vanliga i länder dit svenskar ofta reser på semester, t.ex. Thailand, Egypten, Turkiet och övriga Sydostasien. En annan lanserad teori är att dessa resistensmekanismer ökar på grund av att det konsumeras för mycket antibiotika i onödan vilket leder till att bakterierna utvecklar denna okänslighet för antibiotika. Dock har Sverige haft en mycket positiv trend under de senaste åren då konsumtionen av dessa vanliga antibiotika har minskat med mellan 17 och 27 %.

Sammanfattningsvis indikerar denna studie att de allvarliga komplikationerna har mer än fördubblats under de senaste tio åren.

10.1.1.5 Slutsats

I Stockholm görs 4500 vävnadsprover av prostata varje år. Nästan 1400 män diagnostiseras årligen med prostatacancer till följd av dessa undersökningar. Nästan 60 % av alla män har vid något tillfälle de senaste fem åren tagit ett PSA värde. Trots att PSA är ett vanligt blodprov, som de allra flesta läkare kommer i kontakt med, genomgår bara 67 % av männen i åldrarna 50-59 år en prostatapunktion inom ett år från deras PSA provtagning, trots ett relativt högt PSA.

Genom att utnyttja genetiska markörer kan man identifiera de män med låga värden som har hög risk för prostatacancer. De genetiska markörerna kan också användas för att identifiera en stor andel av de män med ett PSA mellan 4 och 10 ng/mL som inte behöver genomgå vävnadsprovtagning. Under senare år har de infektionsrelaterade komplikationerna efter vävnadsprovtagning ökat markant. Män som ändå behöver genomgå en prostatapunktion måste informeras om att mer än en av 50 män blir så pass sjuka att det anses motiverat att genomföra undersökning för att se om de drabbats av blodförgiftning.

Genom att använda genetiska markörer och en strukturerad uppföljning kan antalet vävnadsprover minskas och därmed också antalet män som drabbas av allvarliga infektioner.

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